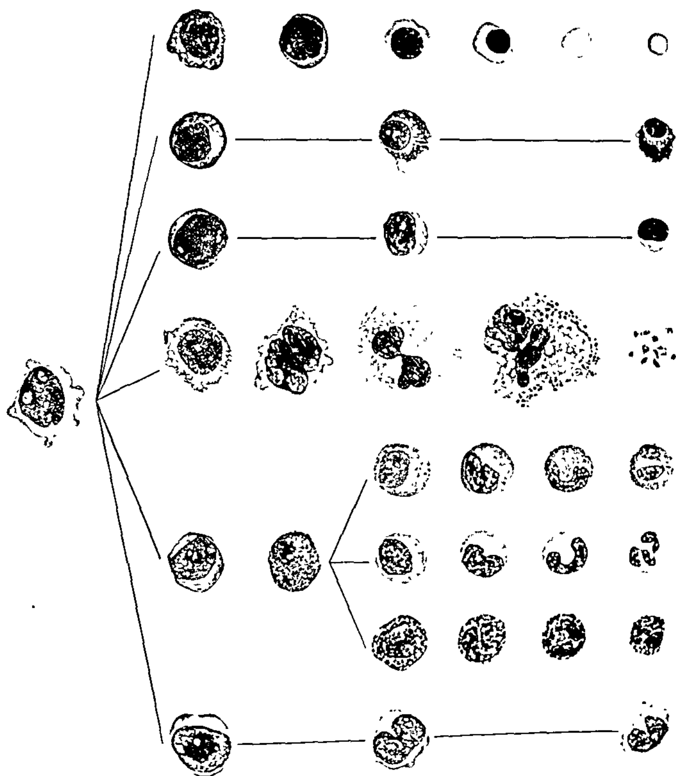
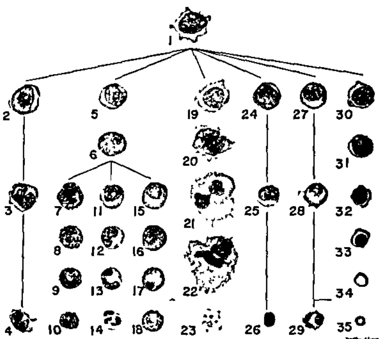
# THE MORPHOLOGY OF HUMAN BLOOD CELLS





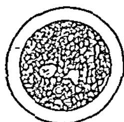
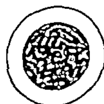

*Frontispiece*

1. Stem cell
2. Monoblast
3. Promonocyte
4. Monocyte
5. Myeloblast
6. Progranulocyte
7. Basophilic myelocyte
8. Basophilic metamyelocyte
9. Basophilic band
10. Basophilic segmented
11. Neutrophilic myelocyte
12. Neutrophilic metamyelocyte
13. Neutrophilic band
14. Neutrophilic segmented
15. Eosinophilic myelocyte
16. Eosinophilic metamyelocyte
17. Eosinophilic band
18. Eosinophilic segmented
19. Megakaryoblast
20. Promegakaryocyte
21. Megakaryocyte
22. Metamegakaryocyte
23. Thrombocytes
24. Lymphoblast
25. Prolymphocyte
26. Lymphocyte
27. Plasmoblast
28. Proplasmocyte
29. Plasmocyte
30. Rubriblast
31. Prorubricyte
32. Rubricyte
33. Metarubricyte
34. Diffusely basophilic erythrocyte
35. Erythrocyte

(From Miller, S. E : Textbook of Clinical Pathology. 5th Ed. Williams & Wilkins Co , Baltimore, 1955.)

L. W. DIGGS, M.A., M.D.

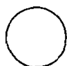
*Professor of Medicine and*
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# THE MORPHOLOGY OF HUMAN BLOOD CELLS


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# PREFACE

THIS ATLAS is written primarily for medical students and student technologists who for the first time are learning about the morphology of normal and pathologic cells, for medical technologists who daily examine blood smears in physician's offices, clinics and hospitals, and for physicians who supervise laboratories. Emphasis is placed on the characteristics of individual cells and on differential morphology rather than on diseases of the blood and blood-forming organs.

Thin smears of human peripheral blood or bone marrow were used Unless specifically stated in the captions, the cells were stained by the Wright's method. The various cells were painted in water color by Dorothy Sturm With the exception of several of the larger plates the paintings were reproduced with an 1800 magnification. The size of the various cells in the color plates is therefore comparable throughout the text.

It is impossible to portray by means of relatively few cells the infinite variations of color, nuclear structure, granules and cytoplasmic morphology of all normal and pathologic cells. The authors have attempted to select cells which are most representative Once selected, a cell was painted or drawn as that particular cell appeared. Diagrammatic representations of morphologic features have been avoided in the color plates. The color plates have been supplemented by black and

white and color photographs, ink drawings, tables and descriptions. All color plates have been placed before text for ready reference and continuity.

The terminology used is that recommended by "The Committee for the Clarification of Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs," sponsored by the American Society of Clinical Pathologists and the American Medical Association (1949–1950). In this terminology the suffix "blast" is reserved for the most primitive cell of a given sequence and the suffix "cyte" for all cells more mature than the "blast." The prefix "pro" is used for the second cell in each developmental sequence. In those sequences in which there are four types of immature nucleated cells, as in the granulocytic and erythrocytic series, the prefix "meta" is applied to the fourth cell.

Because of lack of agreement among hematologists concerning the names for many blood cells, the more common synonyms are given. Descriptive terms based upon morphologic features are used in preference to eponyms.

The bibliography includes selected texts and monographs which are recommended for reading by students No attempt is made to discuss the various theories of cell origin or to give individual credit to the countless workers whose research furnishes the basis for our present concepts. We acknowledge our debt to them and our gratitude for their contributions. Appreciation is also expressed to:

The "Jaycettes" of Memphis, who first financed the painting by Dorothy Sturm of an initial series of cells which serve as visual aids at the University of Tennessee. The development of this series helped us to formulate plans for the atlas and to interest publishers in the project.

Professor Tom Jones of the University of Illinois who encouraged us in the continuation of the art work and who as medical consultant to Abbott and Company made the preliminary arrangements for the first publication of the paintings in color.

Abbott and Company, and particularly Mr. J. S. Dunham, Executive Editor, who published the "Morphology of Blood Cells," first in three issues of "What's New," and later as a monograph. By assuming the initial cost of the color plates and graciously allowing the use of these plates by W. B. Saunders and Company, the publication of the book has been made possible

The staff of the Hematology Laboratory, including Dr. A. P Kraus, Miss Helen Goodman, Mrs Julia Browder, Miss Dorris Shelton, the residents in hematology, Miss Maribette Sifford, Mrs. Janice Perry and Miss Patricia Terry.

Mr. John Dickson, chief medical photographer of the University of Tennessee, who gave freely of his time and skill in making photographs.

The W B. Saunders Company for their numerous courtesies extended to us and for the quality of their work.

L W DIGGS

*April, 1956*

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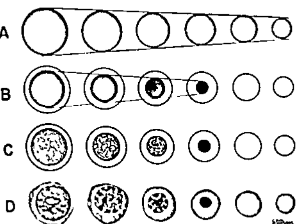
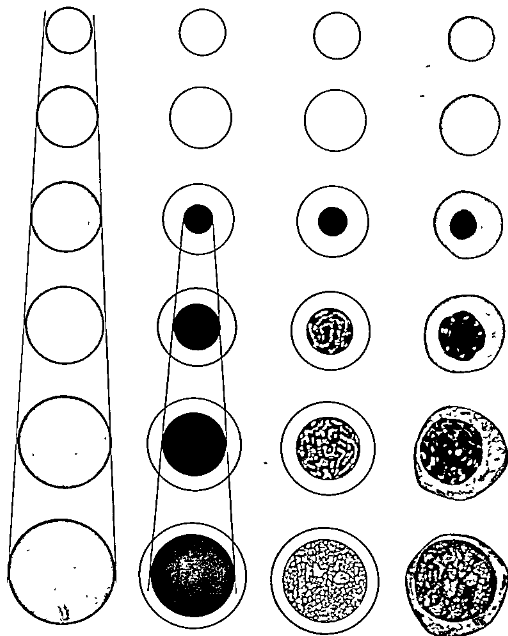



*Plate I.* MATURATION SEQUENCE

A. Cell size and cytoplasm color
B. Nuclear size and color
C Nuclear chromatin structure
D Composite (Left to right: Rubriblast, Prorubricyte, Rubricyte, Metarubricyte, Diffusely basophilic erythrocyte, Erythrocyte)



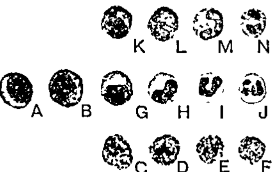
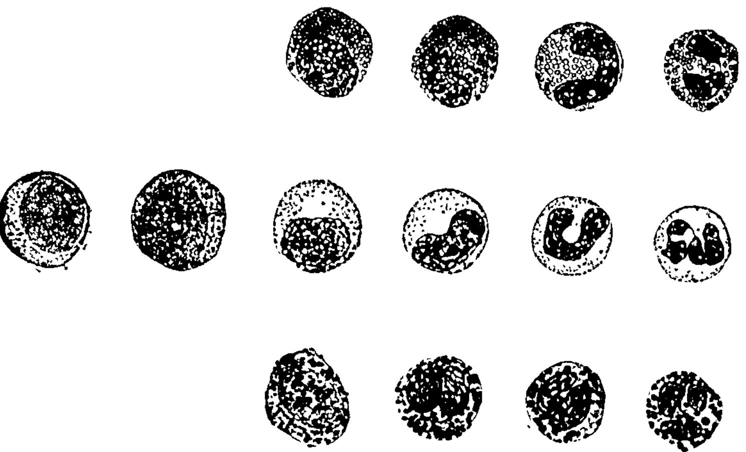



*Plate II.* GRANULOCYTIC (MYELOCYTIC) SYSTEM

A. *Myeloblast*
B. Progranulocyte (promyelocyte)
C. Basophilic myelocyte
D Basophilic metamyelocyte
E. Basophilic band
F. Basophilic segmented
G. Neutrophilic myelocyte
H. Neutrophilic metamyelocyte
I. Neutrophilic band
J Neutrophilic segmented
K Eosinophilic myelocyte
L Eosinophilic metamyelocyte
M. Eosinophilic band
N. Eosinophilic segmented


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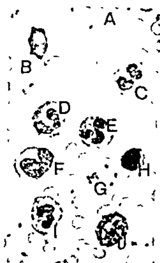
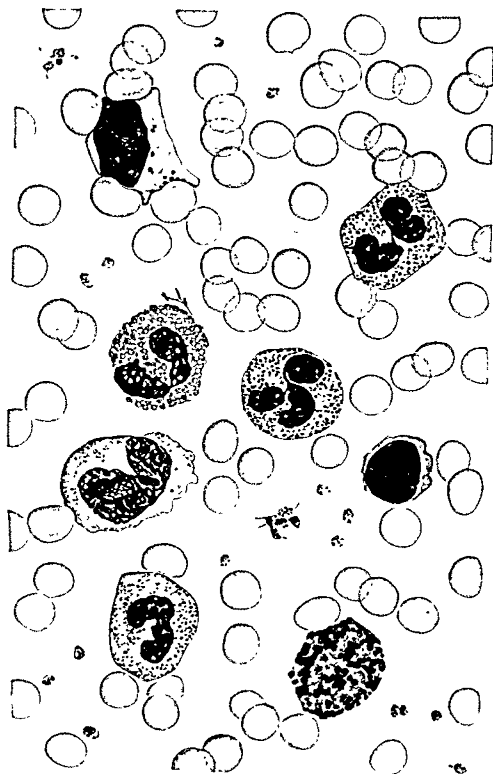



*Plate III.* CELL TYPES FOUND IN SMEARS OF PERIPHERAL BLOOD FROM NORMAL INDIVIDUALS

*The arrangement is arbitrary and the number of leukocytes in relation to erythrocytes and thrombocytes is greater than would occur in an actual microscopic field.*

A Erythrocytes
B Large lymphocyte with azurophilic granules and deeply indented by adjacent erythrocytes
C Neutrophilic segmented
D Eosinophil
E Neutrophilic segmented
F Monocyte with blue-gray cytoplasm, coarse linear chromatin and blunt pseudopods
G Thrombocytes
H Lymphocyte
I Neutrophilic band
J Basophil


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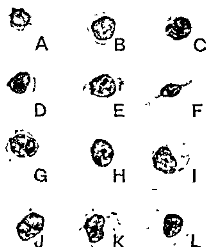



*Plate IV.* LYMPHOCYTES. PERIPHERAL BLOOD

A. Small lymphocyte
B Lymphocyte of intermediate size
C. Lymphocyte with indented nucleus
D. Lymphocyte of intermediate size
E Lymphocyte with pointed cytoplasmic projections (frayed cytoplasm). Typical nucleus
F. Spindle-shaped lymphocyte
G. Large lymphocyte with indented nucleus, unevenly stained dark-blue cytoplasm, and pointed cytoplasmic projections
H. Large lymphocyte with foamy cytoplasm
I. Large lymphocyte with azurophilic granules
J Large lymphocyte with irregular cytoplasmic contours
K Large lymphocyte with azurophilic granules and with indentations caused by pressure of erythrocytes
L Large lymphocyte with a few azurophilic granules

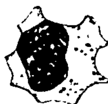
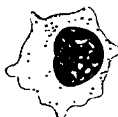










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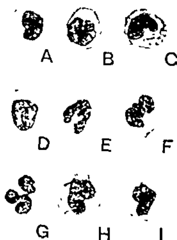



*Plate V.* MONOCYTES PERIPHERAL BLOOD

A. Monocyte with "ground glass" appearance, evenly distributed fine granules and occasional azurophilic granules Vacuoles in cytoplasm

B Monocyte with blue-gray cytoplasm and prominent granules Linear chromatin and lobulation of nucleus

C. Monocyte with prominent granules

D Monocyte with no nuclear indentations and evenly distributed granules

E Monocyte with band type of nucleus, blunt pseudopods, blue-gray color, vacuoles, granules and linear chromatin

F Monocyte with multilobulated nucleus, blue-gray color and irregular shape

G Monocyte with segmented nucleus

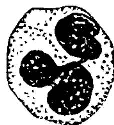
H Monocyte with multiple blunt nongranular pseudopods, nuclear indentations and folds

I Monocyte with vacuoles and with differentiation between nongranular ectoplasm and granular endoplasm











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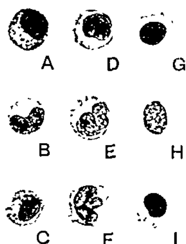



*Plate VI.* COMPARATIVE MORPHOLOGY, NEUTROPHILIC GRANULOCYTES, MONOCYTES AND LYMPHOCYTES

A N myelocyte with mixture of neutrophilic and metachromatic granules

B. N metamyelocyte with pink color and neutrophilic granules

C N myelocyte

D Monocyte with nuclear fold

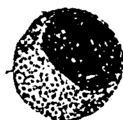
E Monocyte with blue-gray cytoplasm, prominent granules and multilobulated nucleus (brain-like convolutions) and separation of chromatin strands

F Typical monocyte with lobulated nucleus, fine evenly distributed granules and superimposed azurophilic granules, blunt pseudopods, and blue-gray color

G Large lymphocyte with scalloped shape. Absence of folds in nucleus

H Large lymphocyte with fine foamy, nongranular cytoplasm

I Large lymphocyte with azurophilic granules Lumpy nuclear structure











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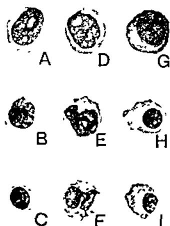



*Plate VII.* LYMPHOCYTIC, MONOCYTIC, AND PLASMOCYTIC SYSTEMS

A Lymphoblast
B Prolymphocyte
C Lymphocyte with clumped chromatin
D. Monoblast
E. Promonocyte
F Monocyte
G Plasmoblast
H Proplasmocyte
I Plasmocyte











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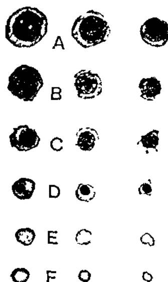



*Plate VIII.* ERYTHROCYTIC SYSTEM*

Left column *Macrocytic erythrocytes (megalocytic, megaloblastic) of the type seen in pernicious anemia and related $B_{12}$-folic acid deficient states.*
Middle column *Normal erythrocytic sequence.*
Right column *Microcytic, hypochromic cells of type seen in iron deficient states*

A Rubriblasts
B Prorubricytes
C Rubricytes
D Metarubricytes
E Diffusely basophilic erythrocytes
F Erythrocytes

* See Table 3 on page 73 for synonyms of the various cells




















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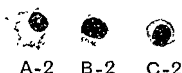




*Plate IX.* COMPARATIVE MORPHOLOGY. *PLASMOCYTES* (A), LYMPHOCYTES (B) AND IMMATURE NUCLEATED RED CELLS (C)

- A1 Plasmocyte with intense blue cytoplasm, eccentric nucleus, clear zone, vacuoles and irregular shape
- A2 Plasmocyte with eccentric nucleus, foamy and fibrillar reddish-blue cytoplasm, clear zone and irregular shape
- B1 Lymphocyte with slightly indented nucleus, unevenly stained bluish cytoplasm
- B2 Lymphocyte with foamy cytoplasm and frayed (hairlike) cytoplasmic margins
- C1 Prorubricyte with reddish-blue cytoplasm
- C2 Rubricyte with polychromatophilia













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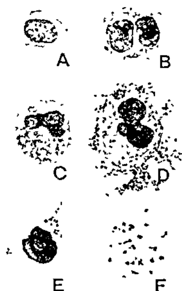



*Plate X.* MEGAKARYOCYTES AND THROMBOCYTES

A Megakaryoblast with bluish foamy marginal cytoplasmic structures

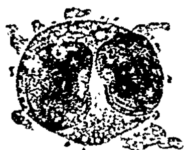
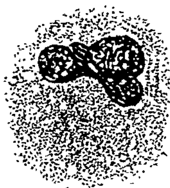
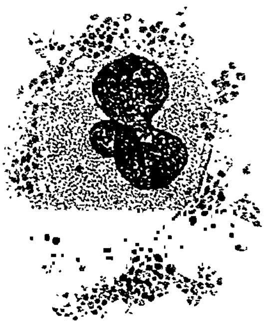
B Promegakaryocyte with granular blue cytoplasm and marginal cytoplasmic structures

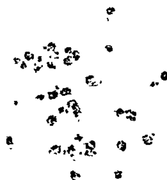
C Megakaryocyte without thrombocyte differentiation and with granular cytoplasm

D Metamegakaryocyte (megakaryocyte with thrombocyte differentiation)

E Megakaryocyte nucleus with attached thrombocytes

F Thrombocytes



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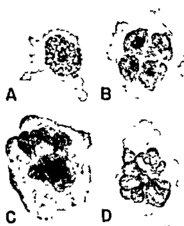



*Plate XI.* MEGAKARYOCYTE VARIANTS

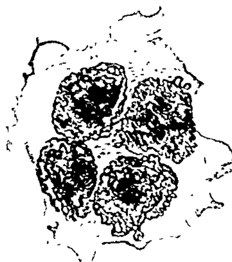
A. Megakaryoblast beginning mitosis

B. Atypical promegakaryocyte with four nuclei Blunt nongranular pseudopods Beginning granule formation

C Megakaryocyte with multilobular nucleus and granular cytoplasm, surrounded by a ring of coarsely vacuolated cytoplasm Blunt dark staining cytoplasmic attachments No thrombocyte formation

D Atypical early megakaryocyte with asynchronism between cytoplasm and nucleus The nucleus is hyperlobulated but cytoplasm is relatively nongranular









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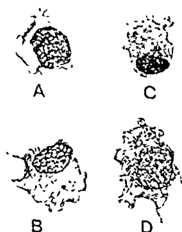



*Plate XII.* MESENCHYMAL CELL AND GRANULAR HISTIOCYTES

A Undifferentiated mesenchymal cell (hemohistioblast)
B Granular histiocyte with few granules
C Granular histiocyte with numerous red granules and rods
D Granular histiocyte with red rodlike structures and granules, many of which are arranged in linear patterns







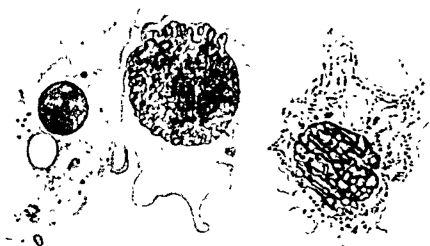


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*Plate XIII.* FIXED TISSUE CELLS OF BONE MARROW

A. Phagocytic histiocyte with vacuoles and phagocytized malarial pigment
B Undifferentiated mesenchymal cell Partial rupture of nuclear membrane
C Granular histiocyte (Ferrata cell)



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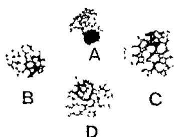
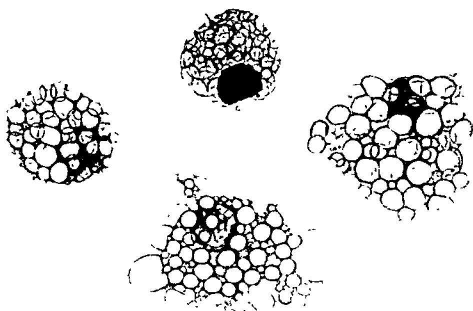



*Plate XV.* MESENCHYMAL CELLS WITH GLOBULAR BODIES

A, B Immature fat cells

C, D Mesenchymal cells with multiple globular structures (? Plasmocytes—? Fat cells)

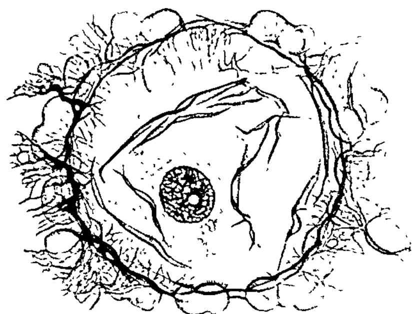


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*Plate XVI.* FAT CELLS

*Upper* Intermediate fat cell with wrinkled membrane, reticular stroma and fibrillar marginal structures

*Lower* Fat cell showing cytoplasmic reticular structure




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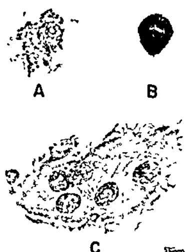



*Plate XVII.* MISCELLANEOUS FIXED TISSUE CELLS

A. Tissue eosinophil
B Tissue basophil
C Osteoclast







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*Plate XVIII* PLASMOCYTE GROUP

*Photomicrograph of group of plasmocytes adjacent to a mesenchymal cell nucleus*



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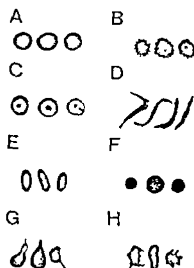



*Plate XIX* PATHOLOGIC ERYTHROCYTES
(*Red blood cells*)

A Normal erythrocytes
B Crenated erythrocytes
C Target cells
D Sickle cells, drepanocytes
E Oval, elliptical cells (ovalocytes, elliptocytes)
F *Spherical cells, spherocytes*
G Pointed cells
H Bizarre forms

















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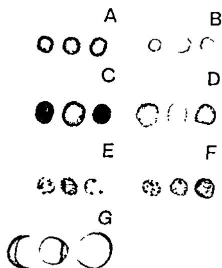
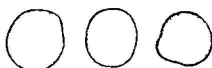



*Plate XX* PATHOLOGIC ERYTHROCYTES
(*Red blood cells*)

- A Normal erythrocytes
- B Erythrocytes showing hypochromia
- C Macrocytic erythrocytes with increased amount of hemoglobin
- D Diffusely basophilic erythrocytes (polychromatophilia)
- E Reticulocytes (brilliant cresyl blue moist preparation)
- F Stippled erythrocytes (punctate basophilia, basophilic aggregation)
- G Semilunar (crescent) bodies






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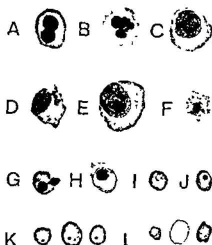
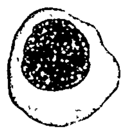
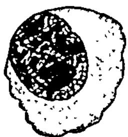



*Plate XXI* PATHOLOGIC ERYTHROCYTES

A Macrocytic rubricyte with double nucleus, pernicious anemia
B Stippled macrocytic rubricyte with karyorrhexis (? polyploidy), pernicious anemia
C. Prorubricyte Asynchronism between 'early chromatin structure and hemoglobin content in cytoplasm, pernicious anemia
D Prorubricyte with nuclear fragments Asynchronism between nucleus and cytoplasm, pernicious anemia
E Macrocytic prorubricyte, pernicious anemia
F. Stippled red cell with degenerated nucleus Atypical metarubricyte
G Metarubricyte with extruding nuclear structure
H Stippled metarubricyte with Cabot's rings and Howell-Jolly body
I Malarial ring
J Thrombocyte on erythrocyte
K Howell-Jolly bodies
L Cabot's rings


















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*Plate XXII.* PATHOLOGIC WHITE CELLS

A. Neutrophil with prominent dark bluish granules ("toxic granules")

B. Neutrophil segmented with vacuoles, a sign of degeneration

C. Neutrophil metamyelocyte with "toxic granules" and cytoplasmic inclusions

D. Degenerated neutrophil with pyknotic nuclei and nuclear fragment

E. Giant neutrophil with multiple nuclei (polyploid neutrophil)

F. Mature neutrophil with round nucleus and coarse, lumpy chromatin (from peripheral blood, granulocytic leukemia)

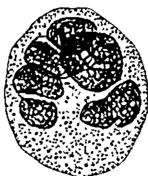
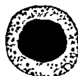
G. Hyperlobulated macrocytic neutrophil ("P A *poly*")

H. Monocyte with phagocytized pyknotic nucleus (to be differentiated from "L. E." cell)

I. Segmented neutrophil with feltlike purple staining mass in cytoplasm, "L. E." cell








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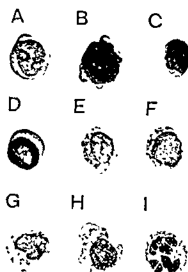



*Plate XXIII* GRANULOCYTIC LEUKEMIA

A Myeloblast with prominent nucleoli and well-defined chromatin structure Deep blue cytoplasm No granules

B Atypical early cell with dark coarse nuclear chromatin structure and blunt vacuolated pseudopods (probably a megakaryocyte)

C. Myeloblast with Auer rod in cytoplasm

D Atypical early cell with large nucleolus and with occasional dark granules

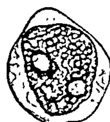
E Atypical progranulocyte with prominent purple granules

F Atypical progranulocyte with fine and coarse granules

G Atypical granulocyte (simulating monocyte) Large cell with relatively large amount of cytoplasm Indented nucleus, intermediate chromatin structure, and nonspecific granules

H Granular histiocyte (Ferrata cell)

I Segmented neutrophil, twinning deformity of nucleus











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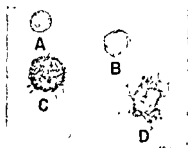



*Plate XXIV.* PEROXIDASE STAIN

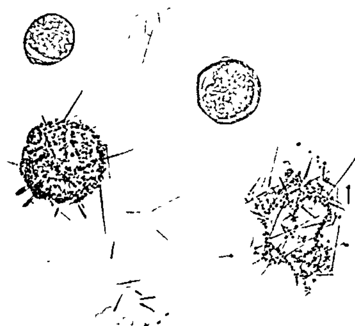
(*Sato and Sekiya*)

A, B The two upper cells (lymphocytes) are peroxidase negative (nongranulocytes).

C, D The two lower cells (neutrophils) are peroxidase positive (granulocytes).

The red cells are laked and appear as *shadow forms*

This stain is of aid in differentiating early cells of the myelocytic-monocytic systems from cells of the lymphocytic system



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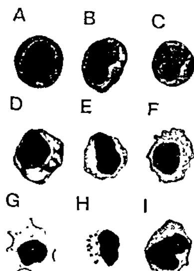



*Plate XXV.* INFECTIOUS MONONUCLEOSIS

A. Primitive plasma-like cell
B, C Atypical plasma-like cells with indented nucleus and early nuclear structure
D Large atypical lymphocyte with unevenly stained bluish cytoplasm
E. Large lymphocyte with foamy cytoplasm
F ? Large lymphocyte—? monocyte
G Large lymphocyte with azurophilic granules and scalloped borders (indented by red cells)
H Atypical lymphocyte with prominent reddish (azurophilic) bodies
I Atypical monocyte











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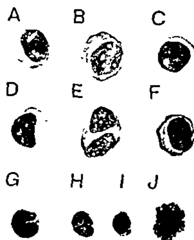
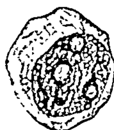



*Plate XXVI.* LYMPHOCYTIC LEUKEMIA

A. Atypical early lymphocyte (? lymphoblast)
B Lymphoblast with prominent nucleoli
C Prolymphocyte with indistinct nucleolus
D. Prolymphocyte with intermediate chromatin structure and rippled appearance of cytoplasm
E Prolymphocyte with double nuclei
F Atypical lymphocyte with clumping of nuclear chromatin, purplish-red nongranular cytoplasm
G Prolymphocyte with deep nuclear cleft
H Lymphocyte, nuclear amitotic division
I Lymphocyte with nuclear fragment
J Smudge









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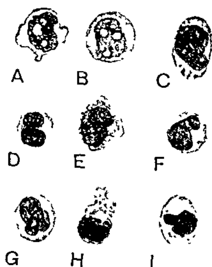
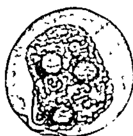



*Plate XXVII.* MONOCYTIC LEUKEMIA

A Atypical monoblast with prominent nucleoli, indented nucleus and blunt pseudopods
B Monoblast with prominent nucleoli
C Atypical promonocyte with vacuoles in cytoplasm, indistinct nucleoli
D Promonocyte with nuclear folds, foamy cytoplasm
E Atypical promonocyte with prominent granules and clear ectoplasm (histiocytic type of monocyte)
F Promonocyte with deeply indented nucleus and granular cytoplasm
G. Monocyte with transparent folded nucleus, granules in cytoplasm
H Monocyte with granules suggestive of granular histiocyte
I Monocyte with phagocytized red cell







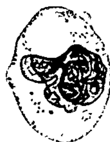












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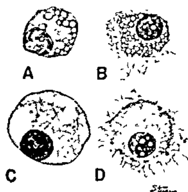
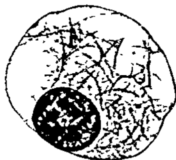



*Plate XXVIII.* PLASMOCYTES

A. Plasmocyte with Russell bodies
B. Plasmocyte with multiple globules (? early fat cell)
C. Plasmocyte showing reticular cytoplasmic structure
D Plasmocyte with Russell-like bodies in nucleus, reticular cytoplasmic structure and red periphery









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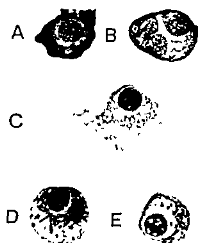



*Plate XXIX* PATHOLOGIC PLASMOCYTES
(*Plasma cell myeloma*)

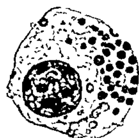
A Plasmocyte with bright red cytoplasm ("flaming" appearance)
B Plasmocyte with three nuclei
C Plasmocyte with nebulous cytoplasmic margins, multiple globules and pink staining homogeneous periphery
D Plasmocyte with red staining crystalline bodies
E Plasmocyte with Russell bodies (eosinophilic globules)











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*Plate XXX.* LIPID HISTIOCYTES

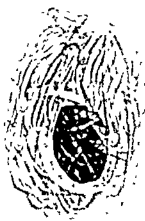
*Left* Lipid histiocyte, found in bone marrow, Gaucher's disease

*Right* Lipid histiocyte, found in bone marrow, Niemann-Pick's disease

*Plate XXXI* BLOOD PARASITES

*Left* Macrophage with phagocytized *Histoplasma capsulatum*

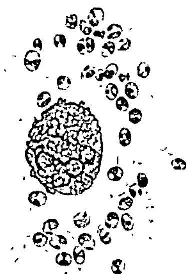
*Right* Macrophage with *Leishmania donovani*





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# Chapter II

# FIXED TISSUE CELLS OF THE BONE MARROW

IN ADDITION to the free blood cells of the peripheral blood and their precursors, there are various types of fixed tissue cells in the bone marrow which form a supporting framework or syncytial net. These stroma cells are numerous but are aspirated with difficulty. In the majority of marrow smears they are so infrequent that they are not included in the routine differential counts. Their recognition is important because they may be involved in proliferative processes and because they may be mistaken for metastatic malignant cells.

The less differentiated fixed tissue cells (Figs. 6, 7) have the potentiality of changing from one form to another under different environmental conditions. Thus the outstretched cell of the marrow syncytium, usually designated as a histiocyte (Gr. *histion,* web + *kytos,* cell) or reticulum cell (L. *reticulum,* little net), may become motile, store lipids or acquire phagocytic properties. It may develop into a blood cell, a lining cell of a blood vessel, a fibrocyte or an osteoblast (Fig. 6). All sorts of transitional forms occur. The names given to the various types of fixed tissue cells (Fig 6; Table 6) are based upon

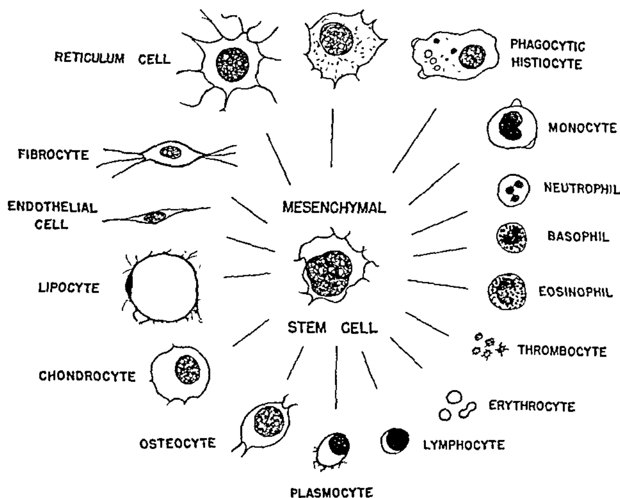



*Figure 6* Diagrammatic representation of fixed tissue and free blood cells originating from mesenchymal stem cell.

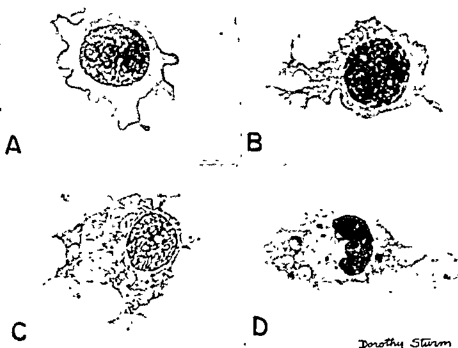



*Figure 7.* Variants of fixed tissue cells of bone marrow

*A*, Undifferentiated mesenchymal cell or stem cell. *B*, Fixed tissue cell with minimal granulations and fibrillar structure (? prohistiocyte). *C*, Granular histiocyte. Note linear type of granules *D*, Phagocytic histiocyte (wandering tissue macrophage) (From Miller, S. E., Textbook of Clinical Pathology 5th Ed Williams & Wilkins Co, Baltimore, 1955 )

**Table 6. Types of Fixed Tissue Cells in Hematopoietic Organs**

| MORPHOLOGY | TERMS |
|---|---|
| Without distinctive morphologic features | Undifferentiated mesenchymal cell (stem cell, primitive cell, hemohistioblast) |
| Granules in cytoplasm | Tissue neutrophil (granular histiocyte, Ferrata cell)<br>Tissue eosinophil<br>Tissue basophil |
| Globules of fat in cytoplasm | Fat cell (lipocyte) |
| Phagocytized particles in cytoplasm | Tissue macrophage (phagocytic histiocyte, reticuloendothelial cell) |
| Distinctive fibrils | |
| Argentophil fibrils | Reticulum cell |
| Collagen fibrils | Fibrocyte (fibroblast) |

the fibrils or fibers with which they are associated, the granules, globules and phagocytized particles in their cytoplasm or the anatomic structures such as blood vessels, bone, cartilage, fat or connective tissue in which they are found.

## General Characteristics

Fixed tissue cells in stained smears have irregular shapes and frayed and fragmented cytoplasmic margins, for they are attached to other cells by intercellular cement substances and fibrils. They are torn in the process of aspiration and spreading in a thin film. Traumatic injury to the nucleus may cause the nuclear membrane to be ruptured or distorted and the chromatin structure to become coarse and linear.

Fixed tissue cells vary considerably in size but as a rule are large cells with ample cytoplasm. The nuclei of the more undifferentiated cells are large and become smaller as the cells acquire distinctive morphologic features. The nuclei of fixed tissue cells, in contrast to cells which are ameboid, are round or oval and do not have folds and indentations.

## Undifferentiated Mesenchymal Cell (Hemohistioblast, Reticulum Cell)

The undifferentiated mesenchymal cell, which is considered to be a progenitor of all types of blood and fixed tissue cells of the bone marrow, is a large cell with a large round or oval nucleus which takes a light and predominantly red stain (Plates XII A; XIII, Fig. 7 A). The chromatin strands vary in texture but are usually well defined One or more nucleoli are usually demonstrable. The light bluish nongranular cytoplasm has a homogeneous or delicate reticular structure Cell margins are irregular, and there may be blunt pseudopods and or fibrillar marginal extensions (Plates XII A, XIII; Figs 6, 7 A).

Transitions between the undifferentiated mesenchymal cells and the so-called "blast cells" of the various blood cell types are seen. The name given to a primitive cell is often arbitrary and determined more by association with other cells than by morphologic criteria.

## Granular Histiocyte (Ferrata Cell, Tissue Granulocyte)

One of the cell types, which is encountered in small numbers (less than 1 per cent) in practically every smear of normal bone marrow, is a granular histiocyte which resembles the undifferentiated mesenchymal cell except for the fact that it contains varying numbers of distinct small granules and short rods which tend to be arranged in a linear pattern.

The granular histiocytes vary considerably in size, but as a class are large. They have abundant cytoplasm and a large oval or round nucleus which has a coarse chromatin structure and often one or several prominent bluish nucleoli. The shape is irregular and there may be cytoplasmic projections of varying types (Plates XII B, C, D; XIII; Fig. 7 C). The cytoplasm stains a light blue and has a fine lattice-like structure (Plate XII B; Fig. 7 C) The granules vary in number. They stain varying shades of red to blue, but the majority take a brilliant red or reddish-purple stain Many of the granules are arranged in beadlike chains (Plates XII B, C, D; XIII; Fig. 7 C).

Granular histiocytes with well-developed rods and linear granules are not phagocytic and seldom have vacuoles in their cytoplasm

Granular histiocytes are increased in myelocytic and in monocytic-myelocytic leukemia (Naegeli type of monocytic leukemia) Cells having the morphology of these cells are the predominant cells in the so-called histiocytic leukemias. Granular histiocytes are also increased in the myeloproliferative diseases and in conditions in which there is a maturation arrest of the granulocytic cells, as in agranulocytosis, and in other diseases associated with peripheral neutropenia

Opinion is divided concerning the position of this cell in the sequence of cell development and its relationship to other cells. Ferrata considered this cell to be a multipotent primitive cell or hemohistioblast, while Naegeli, Rohr and the majority of other hematologists have ignored the cell as an entity, holding it to be a traumatized or degenerated granulocyte In the opinion of the authors the cell is not an artefact but a distinct cell type whose function and fate are not known. It is thought to be a tissue granulocyte, a morphologic variant between the undifferentiated mesenchymal cells and granular cells of the myelocytic and monocytic types

## Phagocytic Histiocyte (Macrophage)

Included among the cells called histiocytes are large cells which have digestive vacuoles and/or phagocytized material within the cytoplasm (Plates XIII A; XIV A, B; Fig. 7 D). Some of these cells have prominent blunt and nongranular pseudopods which are a manifestation of their ameboid activity (Plates XIII A, XIV A), while others tend to round up or to be in a resting phase (Plate XIV B). Others have shaggy cell margins which are characteristic of fixed tissue cells (Plates XIII; XIV C, D). The cells may be packed with phagocytized material (Plate XIV C, D), contain only moderate amounts (Plates XIII; XIV A; Fig 7 D), or show no distinctive cytoplasmic inclusions (Plate XIV B). Ameboid, resting and fixed tissue cells which have such a different appearance at different times are not necessarily different cells but phases in the activity of the same or similar cells.

Phagocytic histiocytes as a class have a comparatively small nucleus and a large amount of cytoplasm. The nucleus is usually round or oval and has a coarse linear chromatin pattern Nucleoli are often visible. The cytoplasm is light blue or bluish-gray

Many of the cells appear foamy and full of holes or have vacuoles of varying size (Plate XIV B, Fig 7). The material commonly phagocytized may consist of whole cells, cytoplasmic fragments, nuclei and nuclear fragments, granules, pigment particles, fat globules, bacteria, fungi and parasites

Phagocytized pigment particles may be golden yellow, brown, green, purple, blue or black, depending upon the chemical nature and the size of the particle Pigment-laden macrophages are most frequently seen in patients who have received transfusions and in diseases in which there is a shortened survival time of erythrocytes and an increased rate of hemolysis. In hemochromatosis there is defective iron metabolism with the storage of iron containing pigment (hemosiderin) together with iron-free pigment in the phagocytes of the hematopoietic organs as well as in the parenchymal cells of various organs.

## Fat Cell (Lipocyte)

The morphologic sequence of the development of a fat cell from an undifferentiated mesenchymal cell to the large and mature cell filled with a fused large globule of fat with a flattened and peripherally located nucleus is not well established. The first recognizable fat cells contain multiple small globules of varying size which practically fill the cytoplasm These globules take pastel tints of pink, orange or lavender when stained with Wright's stain In thin preparations the globules may be separated by thin strands of bluish cytoplasm (Plate XV B), but in thicker preparations the globules lie over each other, giving striking circular designs such as would be produced by drawing superimposed delicate blue circles with a small compass (Plate XV A, B). The nucleus is relatively small, round or oval, is eccentrically located and has a variable and nonspecific chromatin pattern. The fat globules often overlie and obscure the nucleus (Plate XV B). The cytoplasmic membrane may be ruptured, causing the globules to appear as free groups with or without an attached degenerate nucleus.

Later stages of the fat cell are manifested

in tissue sections as large chromophobic areas surrounded by other cells but are not seen in thin smears as single cells for they are ruptured in the process of aspiration and spreading. The fat escapes from the traumatized cell and the cell stroma and membrane are not recognizable. On searching one may find an intermediate form of the fat cell (Plate XVI). The fixed tissue character of these cells is revealed by multiple fibrils which extend outward from the margin.

In addition to the true fat cells described above, which are thought to synthesize fat within the cell from nutrient substances absorbed in a soluble form from the blood, there are cells which contain globules of fat which are thought to be phagocytized as visible globules and stored by various types of fixed tissue cells (lipophages, fat-storage cells). Transitions between true lipocytes and various fat-laden mesenchymal cells are demonstrable and may be impossible to differentiate.

Plasmocytes with multiple eosinophilic globules or Russell bodies may closely simulate the morphology of true fat cells and on morphologic grounds cannot be distinguished without the use of special stains (Plate XV C, D)

## Tissue Basophil (Mast Cell)

Tissue basophils or mast cells appear in small numbers in bone marrow smears of normal individuals (Plate XVII). They vary from 15 to 40 micra in diameter. They often are elongated or have spindle and irregular shapes The nucleus is usually eccentric The side of the cell in which the nucleus is present is likely to be rounded and the opposite portion of the cell tapered The nucleus is small, round or oval and has nonspecific chromatin structure The cytoplasm is filled with intensely stained violet blue granules The granules are round and are of fairly uniform size (0.1 to 0.3 micra) Sometimes the bluish cytoplasm is visible, but ordinarily the cell is so packed with dark granules that the cytoplasm is not seen The granules frequently overlie the margins of the seemingly pale nucleus or may partially or completely obscure the nucleus.

It is thought that the tissue basophils are derived from undifferentiated mesenchymal cells, but transitional stages between primitive cells and highly differentiated forms have not been well documented.

The granules of the tissue basophils are thought to be manifestations of a secretory function. The mast cells are concerned with the synthesis and secretion of heparin and histamine. The relationship of the tissue basophils is not known, but there are evidences of similarity in metabolic and secretory function (Valentine et al.: Blood *10*:154, 1955).

Tissue basophils are increased in certain pancytopenic states and in some cases of myelosclerosis. In conditions in which these cells are increased there is often an associated hemorrhagic tendency. These cells may be demonstrated in certain marrows in which there is no hemorrhagic correlation.

## Tissue Eosinophil

One occasionally sees in bone marrow smears large cells with elongated and tapering cytoplasmic extensions and containing typical eosinophilic granules of the type seen in the eosinophils of the circulating blood. The nucleus of such cells, instead of being indented or lobulated, resembles that of the other fixed tissue cells, being spherical or oval and having a coarse reticulum chromatin pattern and often having nucleoli (Plate XVII) The relationship of this cellular type to the eosinophils of the blood is not known.

## Osteoblast, Osteoclast and Osteocyte

### OSTEOBLAST

Osteoblasts are unicellular elements of the bone marrow associated with bone formation They are readily demonstrable in tissue sections of normal marrow They seldom appear

in bone marrow smears, for these fixed tissue cells are adherent to the bony trabeculae and are aspirated with difficulty. In marrow smears they may appear as isolated cells but frequently are in small groups. They are found most frequently in marrow smears of young children, in Paget's disease and in metastatic malignant lesions in which there is increased formation of new bone.

The morphologic features of the osteoblast (Figs 8, 9) are quite similar to those of plasmocytes Like the plasmocytes, osteoblasts have irregular and elongated shapes, frayed cytoplasmic margins, round eccentric nuclei and bluish cytoplasm which is often irregularly stained and has a bubbly appearance. There is an area in the cytoplasm which takes a distinctly lighter stain than the rest of the cytoplasm

Osteoblasts, like plasmocytes, vary considerably in size. Osteoblasts as a rule are larger. One or more nucleoli are perceptible in the nuclei of osteoblasts but are not seen in the nuclei of mature plasmocytes. The concentric reticular cytoplasmic structures which are parallel with the nuclear membrane in the plasmocytes are not observed in osteoblasts Perhaps the most important differential point is that the light area in the cytoplasm in the osteoblast is not immediately adjacent to the nucleus as in the plasmocyte but is an appreciable distance from the nucleus (Fig. 8).

## OSTEOCLAST

Osteoclasts (Gr. *osteon*, bone + *klastos*, broken) are large multinucleated cells of the bone marrow which are most conspicuous in areas in which bone tissue is being absorbed in contrast to uninuclear osteoblasts which are predominant in areas in which there is formation of new bone. The authors consider the osteoclast to be a polyploid giant form of the osteoblast. The nucleus of this divides and redivides without an associated division of the cytoplasm.

The osteoclast is a large irregular shaped and usually elongated cell with multiple

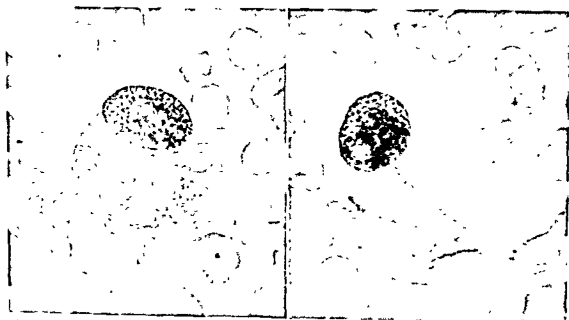


*Figure 8* Photomicrograph Bone marrow smear Osteoblasts Eccentric nucleus Foamy cytoplasm Irregular shape similar to plasmocytes Light area in cytoplasm not adjacent to nucleus Coarsely granular nuclear structure (Courtesy of Dr Dorothy Sundberg, Minneapolis, Minn )



*Figure 9.* Microphotograph. Group of osteoblasts. Bone marrow smear. Rouleaux formation

round oval nuclei which are of approximately the same size (Plate XVII). The nuclei are separate and are distributed haphazardly within the cytoplasm. Nucleoli may be present in some of the nuclei. The abundant blue cytoplasm has a granular or ground glass appearance

Osteoclasts are similar to megakaryocytes in that they both are large and have multiple nuclei and a bluish granular cytoplasm. The nuclei of megakaryocytes are multilobulated or are connected by nuclear strands, whereas the nuclei of osteoclasts are usually separated Megakaryocytes often have peripheral attachments of foamy cytoplasm, marginal vacuoles or attached thrombocytes at the periphery, whereas these structures are not apparent in the osteoclast (*Plate XVII*)

### OSTEOCYTE

The term "osteocyte" is used for the fixed tissue cells which occupy the elliptical spaces between the lacunae of compact bone. These cells are not seen in smears of material aspirated from bone The origin and function of osteoblasts, osteoclasts and osteocytes and *their relationship to each other is not settled.*

## Other Fixed Tissue Cells

### FIBROBLAST (FIBROCYTE)

Fibroblasts, although plentiful in the normal bone marrow and increased in certain pathologic conditions, are not readily aspirated They are not seen, or at least not iden-

tified, as specific cells in Wright's stained smears. In tissue sections the fibroblasts have an elongated spindle-shaped nucleus and branching pointed processes (Fig. 6). The nucleus is usually elongated or oval.

### RETICULUM CELL

Reticulum cells which have argyrophilic fibers when stained by silver stains are not distinguishable from undifferentiated mesenchymal cells in Wright's stained preparations. Many consider that the collagen fibers of the fibroblast are more mature variants of the reticular fibers.

### ENDOTHELIAL CELL (ANGIOCYTE)

Cells which line the vascular channels are occasionally recognizable in tissue fragments by their elongated shape, flattened nucleus and anatomic pattern but are not identifiable or do not appear as specific isolated cells in marrow spreads (Fig. 6).

### IMMATURE CELLS OF THE LEUKOCYTIC, THROMBOCYTIC AND ERYTHROCYTIC SYSTEMS

Stem cells and intermediate forms of the blood cells are "fixed" in the sense that they do not under normal conditions escape into the peripheral blood On the other hand, the immature cells of the type destined to produce blood cells are less "fixed" than are fibroblasts, osteoblasts and fat cells and are readily aspirated. The morphology of the fixed tissue forms of the leukocytic, thrombocytic, and erythrocytic series are given in Chapter I.

### PLASMOCYTE (PLASMA CELL) (PLATE XVIII)

Plasmocytes are described in Chapters I and V.

# Chapter III

# NORMAL AND ABNORMAL MITOSES

## Normal Mitosis

Blood cells are derived from other cells by the process of mitotic division Some of the stem cells, after division, remain undifferentiated. Others undergo maturation changes During the process of maturation they may redivide, producing cells of like morphology. These cells may in turn reproduce at the same level of development, or may mature without further division, or may partially mature and redivide at a more mature level The majority of mitoses occur at the "pro" or intermediate levels, and less frequently at the "blast" and "meta" stages of cell development. The potentiality of cell reproduction is lost after the nucleus has degenerated and has become pyknotic.

Mitotic figures are not observed in the smears of peripheral blood of normal individuals Their presence in the circulating blood is a sign of abnormality and presumptive evidence of malignancy, although such forms may occasionally be observed in nonmalignant proliferative processes

In bone marrow smears of normal individuals, the cells in mitosis constitute from 0.1 to 1 per cent of the nucleated cells. Mitotic

figures are more numerous among nucleated red cells than in cells of the leukocytic series. A percentage of mitotic figures greater than 1 is indicative of abnormal cell reproduction. An incidence of mitotic figures less than 1 in 1000 nucleated cells is a sign of hypoplasia or aplasia.

Normal human somatic cells, including the blood cells, contain in their nuclei an average of 24 paired chromosomes or a total of 48 chromosomes. In the first stage of mitosis the chromatin strands which were previously intertwined and indistinct become arranged in rodlike or bent structures which are thick, short, and stain intensely. Each thickened and condensed chromosome splits longitudinally. The nuclear membrane disappears. The divided chromosomes migrate, one half going to one pole and the other half to the opposite pole. The equally divided and separated nuclear particles coalesce and a new nuclear membrane forms around each chromatin mass. At the time of karyokinesis, the cytoplasm has a mottled or granular appearance. Following or associated with the division of the nucleus, the cytoplasm constricts and divides into approximately equal parts, each unit of which contains one of the daughter nuclei. Each cell after fission contains the same number and type of chromosomes as the parent cell and has similar cytoplasmic features.

During the process of karyokinesis and cytokinesis the nuclear and cytoplasmic particles are in a stage of violent agitation. There is rapid protrusion and retraction (*bubbling*) of the margins of the cell. Cells smeared and rapidly dried during mitosis often become fixed in irregular shapes and have multiple cytoplasmic tags. Following mitosis there is a quiescent period during which the cell grows with or without demonstrable maturation changes.

## Abnormal Mitosis

One of the most common abnormalities of mitosis is the division of the nucleus without corresponding separation of the cytoplasm, producing a cell with two distinct nuclei (Plates XXIII I; XXVI E). In this type of *twinning deformity*, each of the nuclei has a normal number of chromosomes and a normal chromatin pattern The cell containing the two nuclei is approximately twice the size of a normal uninuclear cell and maintains its characteristic granules, organoids and staining properties.

Another type of polyploid cell is one in which the nucleus undergoes the preliminary phases of mitosis with an increase in chromatin number, but the nuclear membrane remains intact and no division occurs. This form of polyploidy is known as endomitosis (Fig. 32). The nucleus in such cells is abnormally large and the total size of the cell is increased in comparison with cells of the same type which are dividing normally. Sometimes the cell may partially, but not completely divide, producing lobular forms or nuclei with deep fissures or cleavage lines of varying length (Plate XXVI G, H; Fig. 26).

Multipolar mitosis leads to the formation of cells with varying numbers of nuclei, some of which may be of different sizes. Multiple nuclei may also be produced by the mitosis of one nucleus of a pair, without the participation of the other nucleus in the mitotic process.

Nuclei which are produced at the time of abnormal mitosis may have less than the normal number of chromosomes. These cells as a rule are smaller than normal. During the process of mitosis, one or more chromosomes may fail to be included in the reconstructed nuclear membrane and appear in the cytoplasm as stray nuclear fragments (Plate XXVI I) Sometimes the nuclear material appears to be herniated through the nuclear membrane and appears as mushroom-like nuclear protrusions into the cytoplasm (Fig 35).

Giant polyploid cells with multiple nuclei (Plate XXII E) are not always a sign of abnormality, for such forms are normal in megakaryocytes and osteoclasts (Plates XI, XVII). Double nuclei are frequently found

in plasmocytes and in nucleated red cells. Polyploidy in cells of the myelocytic, lymphocytic and monocytic series is rare in conditions other than malignancy but is not necessarily significant.

In pathologic conditions there may be alterations in the frequency and speed of cell division in relation to the periods of growth and the rate of growth between cell division. Cells may divide rapidly, producing small cells called "microcytes" or "microblasts." These diminutive cells often have jagged and irregular margins and spindle shapes, giving them a spider-like appearance. Since they are small and have a round nucleus and small amounts of bluish cytoplasm, they are frequently mistaken for small lymphocytes.

Dissociation in the rate of mitosis and the growth of cells may, as in pernicious anemia and myeloproliferative diseases, lead to the production of large cells with large nuclei which have a normal number of chromosomes (Chaps. IV and V).

## Amitosis

"Amitosis" is a term which has been applied to the division or separation of a portion of the nucleus without a splitting of the chromosomes and the halving of the chromosome number. The term "amitosis" should be reserved for the fragmentation of cells as a result of senile and degenerative changes or the disruption of cells and nuclei as the result of mechanical trauma. There is meager evidence that human cells divide by the amitotic process and produce new cells which are in turn capable of reproducing their kind.

One occasionally sees in blood and bone marrow preparations cells which appear to be dividing without the usual evidence of mitosis such as chromosomal thickening, realignment of chromosomes, and spindle formation. The nuclei of these cells may have fissures or sharply defined cleavage lines. Many have interpreted such morphologic changes as evidence of amitotic division. A more likely explanation is that mitosis started in a defective or abnormal cell. Before the nuclear and cytoplasmic division was completed, the process was arrested, after which the nucleus degenerated. The cell is seen hours after the mitotic phase is over and after the mitotic figures have been masked by the pyknotic changes such as occur in all aging cells.

Chapter IV

# ABNORMALITIES IN THE MORPHOLOGY OF ERYTHROCYTES

## Non-nucleated Erythrocytes

### *Variations in Size (Anisocytosis)*

#### MICROCYTE

Microcytes are erythrocytes which have a diameter less than 6 micra and a corpuscular volume less than 75 cubic micra. The majority of microcytic erythrocytes originate in the bone marrow from nucleated progenitors which are smaller than normal and which are deficient in hemoglobin Microcytes may also be produced by the fragmentation of the cytoplasm of nucleated or of non-nucleated erythrocytes, producing misshapen cells or "schizocytes"

Anemias in which the average red cell diameter or mean corpuscular volume is less than normal are known as microcytic anemias There is often an associated decrease in the mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. The majority of cells in anemias of the microcytic type are lightly stained and have prominent areas of central achromia (Plate XX B).

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**Table 7. Microcytic and Macrocytic Anemias**

MICROCYTIC ANEMIA

Chronic blood loss
Defective intake, absorption or metabolism of iron
Simple achlorhydric anemia
*Hereditary leptocytosis (thalassemia)*

MACROCYTIC ANEMIA

Pernicious anemia
Megaloblastic macrocytic anemia (pernicious anemia type) due to or associated with:
- Nutritional deficiency (tropical macrocytic anemia)
- Infancy
- Pregnancy
- Tropical sprue
- Nontropical sprue (idiopathic steatorrhea)
- Pellagra
- Gastric lesions, including gastrectomy, carcinoma, polyp
- Intestinal lesions; including partial obstruction, short circuiting operation, fistula, chronic infection, celiac disease
- Diphyllobothriasis and other intestinal parasitic diseases
- Leukemias under treatment with anti-folic acid drugs

Megaloblastic macrocytic anemia of unknown cause (achrestic anemia, refractory macrocytic anemia)
Nonmegaloblastic macrocytic anemias.
- *Diseases characterized by increased number of reticulocytes,* including hemolytic anemias and recovery from marrow aplasia
- Hypoplastic anemias
- Myeloproliferative diseases
- Malignancies involving the bone marrow (myelophthisic anemias)
- Hypothyroidism (myxedema)

MACROCYTE (MEGALOCYTE)

Red cells having a diameter greater than 8 micra and a corpuscular volume greater than 95 cubic micra are known as macrocytes (Plate XX C; Fig. 10 E). Large erythrocytes are the progeny of macrocytic nucleated red cells (megaloblasts, macroblasts). (See under Megaloblastic Erythrocytes.)

Anemias in which the diameter of the average cell is greater than normal or the mean corpuscular volume is increased are called macrocytic or megaloblastic anemias (Table 7).

## *Variations in Shape (Poikilocytosis)*

CRENATED ERYTHROCYTE

One of the normal variants of erythrocyte morphology is a cell with varying numbers of blunt cytoplasmic projections or multiple points (Plate XIX B). These cells are most frequently seen in smears of blood which dry slowly. They are likely to be increased in thick smears and in smears made in cold rooms and in an atmosphere in which the humidity is high, and are unlikely to be present in very thin smears which are rapidly dried It is thought that the puckering of the surface occurs during the transformation of the cell from a biconcave disk to a spherical shape and that the irregularities disappear when the cells become spherical The process reappears when the cell reverts to a biconcave disk Cells which are crenated are thicker than normal cells and have a decreased diameter

No significance is to be attached to the finding of crenated cells, for the abnormality of shape is commonly seen in blood smears of normal individuals and may be marked in one smear and absent in others made from the same patient. Recognition of crenation is important, for the pointed projections at the top of and beneath the cells may be mistaken for stippling or for granulofilamentous substances (reticulocytes). The points or projections on the surface of the cell have highlights and shadows as the focus is changed, whereas structures within the erythrocyte lack this feature.

ACANTHOCYTE

Erythrocytes with long and pointed or thornlike projections of the surface are called "acanthocytes" The prominent spinous processes of these deformed cells are irregularly spaced and vary in length and width. These cells are differentiated from crenated cells by the longer and greater variability in the size of the cytoplasmic projections of the acanthrocytes in contrast to the relatively uniform and short pointed or blunt projections of the crenated cells. The abnormal shape of the acanthocyte is inherent in the structure of the cell and is demonstrable in approximately the same percentage in all preparations, whereas the crenated cells may be present in one preparation of a given patient and absent in others, depending upon the conditions to which the cell is exposed after removal from the body.

BUR (BURR) CELL

The term "bur" has been applied to the misshapened erythrocytes which have irregularities of surface and spicules which resemble the seed pod of certain plants, such as the burdock or cockle bur These cells are defectively formed cells and are not produced, as are crenated cells, by extraneous factors. The cells described by some authors as "bur" cells and by other authors as "acanthocytes" have similar morphologic characteristics. The clinical significance, specificity and the hereditary features of the pointed cells have not been established definitely and require further study.

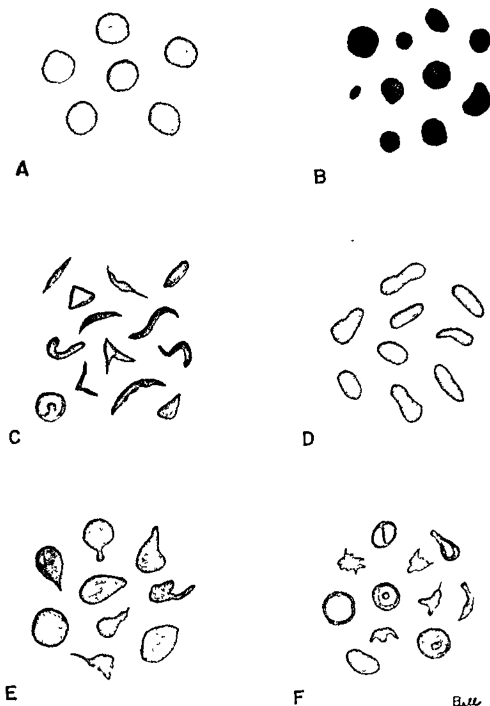



*Figure 10.* Drawing showing comparative size, shape and intensity of staining in erythrocytes *A*, Normal *B*, Spherocytic anemia *C*, Sickle cell anemia *D*, Hereditary ovalocytosis *E*, Untreated pernicious anemia. *F*, Thalassemia

## OVALOCYTE AND ELLIPTICAL CELL

Ovalocytes are egg-shaped or elongated erythrocytes with rounded ends (Plate XIX E; Figs 10, 11). The degree of elongation varies in different cells (Figs 10, 11). The more drawn out forms may be 3 to 4 times greater in length than in width. A small area of central pallor appears in many of the cells, and in iron-deficient states there may be marked central pallor. Although elliptical cells characteristically have rounded ends, they are subject, as are normal cells, to crenation and to pointed and bizarre-shaped transformations (Plate XIX H). Tapering ends and points at one end are fairly common variants, but extremely long and intensely stained double pointed forms bent in the middle (sickled forms) are uncommon.

Elliptical shapes apparently develop days after the cell has been delivered into the circulating blood, for nucleated red cells and reticulocytes rarely reveal the oval cell type of distortion

Oval cells are found in numerous types of anemia and are not diagnostic They are particularly common in blood loss and iron deficiency anemia and in sickle cell anemia. The oval or elliptical cell trait may be inherited as a dominant characteristic This is usually a relatively benign condition which is not necessarily associated with anemia or signs of increased red cell destruction, but which may be characterized by a mild anemia of the hemolytic type.

In sealed moist preparations in conditions other than sickle cell disease the elliptical cells may become more distorted and reveal blunt marginal protrusions, but are not transformed into long, pointed forms In patients with sickle cell disease the oval and elliptical cells in sealed moist preparations develop sharp pointed protrusions, which extend outward from the ends of the original elliptical cells.

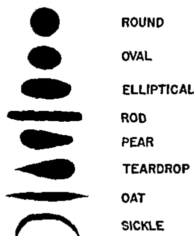



*Figure 11* Common variants of shape in erythrocytes

## LEPTOCYTE

The term "leptocyte" is applied to a cell thinner or flatter than normal. Such cells appear pale and have an abnormally large area of central achromia. In contrast to spherocytes, which have a decreased resistance to hypotonic saline solutions, leptocytes are more resistant than are normal cells to hemolysis in hypotonic solutions, for they are able to swell and increase their volume to a greater extent before the cell membrane is placed under tension Leptocytes are present in conditions in which there is diminished hemoglobin synthesis as in thalassemia and in iron deficiency anemias

The term "leptocyte" has been employed as a synonym for target cell, but this use is to be discouraged, for leptocytes may or may not have an elevated central area

## TARGET CELL (MEXICAN HAT CELL)

Target cells are erythrocytes which, instead of having the usual biconcave disk shape, have an elevation in the center, surrounded by a depression which in turn is bounded by the thickened rim of the cell (Plate XIX C).

The cell appears to have a dark central area in the middle, resembling the bull's eye of the target, encircled by a light area and another ring of darker color. Often the dark area in the center of the cells does not appear as an island but may be in the form of a peninsula extending inward from the rim (Plate XIX C).

Target cells are seen in small numbers in normal blood and in many apparently unrelated conditions such as obstructive jaundice and iron deficiency anemias They are numerous in conditions in which there are abnormal types of hemoglobin, such as hemoglobin C, D, E and S. The target cells are more numerous in smears from patients with homozygous sickle cell anemia (S-S) than in the heterozygous sickle cell trait (S-A), and more common in homozygous C disease (C-C) than in the heterozygous form (C-A). Target cells are also increased in thalassemia minor and major, and in sickle cell-hemoglobin C disease (S-C).

## SPHEROCYTE (SPHERICAL CELL)

Spherocytes are erythrocytes which are thicker than normal. They stain more intensely than do normal cells and usually have a less conspicuous area of central achromia than do normal cells (Plate XIX F; Fig. 10 B). In thin blood smears the spherical cells vary in size and shape, but the majority have a perfectly circular shape and a reduced cell diameter. The mean corpuscular volume in conditions characterized by spherocytosis is essentially normal except in those situations in which there is a marked increase in reticulocytes, in which case there may be an increase in the volume of the average cell. The abnormal thickness of the cells can be visualized and measured by observing the cells in stacked columns in moist preparations. Spherical cells in rouleaux formation are irregularly stacked and do not form even coinlike aggregates as do normal cells of discoid shape.

Spherocytes are found in hereditary spherocytosis and in many types of acquired *hemolytic anemia*. They are more easily hemolyzed than are *normal cells in hypotonic* salt solution and in an *environment* in which the *p*H of the blood is lowered. They have a decreased survival time in the circulation and are more *readily destroyed* when subjected to mechanical trauma. In conditions in which spherocytes are found in blood smears there is often an associated rise in reticulocyte count and signs of increased rate of erythrocyte destruction.

## SICKLED ERYTHROCYTE (SICKLE CELL, MENISCOCYTE, DREPANOCYTE)

Sickled cells are elongated and narrow erythrocytes which have sharp points at both ends. The most typical shape is the crescent form which resembles the cutting blade of a scythe or sickle. In addition, there are other variants which include the long straight cell with double points (oat cell) and "L," "S" and "V" forms (Figs. 10, 11, 12; Plate XIX, D). The hemoglobin in sickled cells appears to be more concentrated than in nonsickled erythrocytes. Some of the sickled cells are vacuolated, and have scalloped and wavy marginal lines, giving them a moth-eaten appearance.

*Sickled cells* are *demonstrable in peripheral blood smears* in homozygous sickle cell anemia (S-S) and in sickle cell-hemoglobin C disease (S-C) but are not present in smears of blood from individuals with the heterozygous sickle cell trait (A-S).

The percentage of sickled cells in ordinary smears varies in different patients with sickle cell anemia, but tends to be fairly constant in a given patient on successive examinations. The number of long and double pointed forms is not *correlated with the severity of* the anemia. In many patients with severe sickle cell anemia, it may be difficult to find typical and diagnostic sickled cells, whereas in other patients the crescent forms may con-

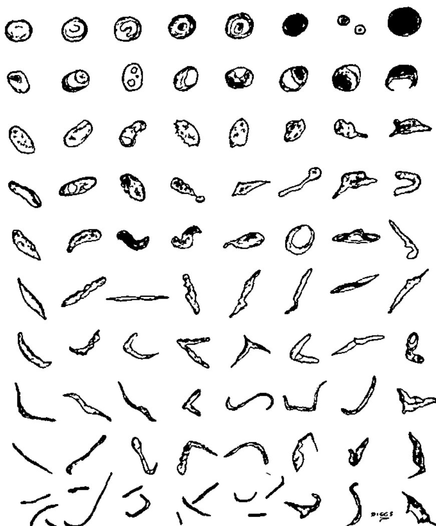


*Figure 12* Drawing of erythrocytes from blood smears of patients with sickle cell anemia

stitute 10 to 30 per cent of the erythrocytes In sickle cell-hemoglobin C disease the target cells are prominent, but there are few sickled cells

In *sealed moist preparations* or in moist preparations of blood mixed with a reducing agent such as sodium metabisulfite, characteristic multipointed forms are demonstrable, provided the patient has sickle cell disease in any of its variant forms. These forms by usage are designated as "sickled cells" but the term "sickle" in a descriptive sense is not applicable. These cells have multiple points and flat sheetlike and scalloped projections which resemble the fins of a fish (Fig. 13)

The spiculate and fish-fin shapes of erythrocytes in sickle cell disease are due to the presence of abnormal sickle cell hemoglobin (Hgb S) which is insoluble in the deoxygenated state and forms long and pointed crystals. These intracorpuscular crystals distort the membrane of the cell The number of sickled cells varies in different preparations depending on the environment to which the



*Figure 13.* Morphologic variants of sickle cells in unstained sealed moist preparation (Courtesy E Vaubel, Med u Kinderk, *52* 504–542, 1937 )

cells are exposed. If the conditions are suitable, there will be 100 per cent sickled cells in the sickle cell trait as well as in sickle cell anemia. Under the conditions of the moist preparation as they are prepared in everyday practice there may be no demonstrable sickled cells in one coverslip chamber and a large percentage in another, both made from the same patient and under apparently similar conditions. A few typical sickle cells are as significant as a high percentage in establishing morphologically the existence of hemoglobin S.

The rate of crystal formation and the length of the crystals is greater in sickle cell anemia in which there is a preponderance of sickle cell hemoglobin, small percentages of fetal hemoglobin, and no adult hemoglobin than in the sickle cell trait in which there is a preponderance of normal hemoglobin and relatively small amounts of sickle cell hemoglobin

Sickled cells in *sealed moist preparations* made from fresh blood will, on exposure to air, immediately revert to their biconcave shape, for the sickle cell hemoglobin on reoxygenation goes back into solution and the crystalline form is lost. Cells that have been maintained for long periods of time in the crystalline state lose their ability to revert to normal shape again and become irreversibly sickled It is believed that the erythrocytes which maintain their elongated and pointed forms on exposure to the air, as in *stained blood smears*, are cells which have undergone the sickling process in the capillaries or the sinusoids of the spleen, bone marrow and other viscera as a result of hypoxia These cells have lost their ability to revert completely to the biconcave shape, although they become less pointed and more spherical when exposed to oxygen.

In rapidly sealed moist preparations of *aspirated bone marrow* material from patients with sickle cell anemia, many of the erythrocytes are extremely long and cylindrical (Fig. 14) In similar preparations from patients with the sickle cell trait without anemia,

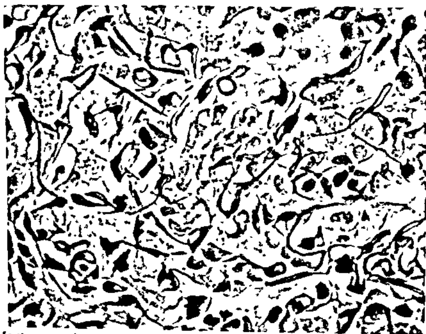


*Figure 14.* Moist unstained preparation of bone marrow Sickle cell anemia Elongated, pointed erythrocytes

elongated and lanceolate forms are not seen. This is direct evidence of *in vivo* differences between the shape of erythrocytes in sickle cell anemia as contrasted with the sickle cell trait.

### OAT CELL

Oat cells are elongated erythrocytes with sharp points at either end. They are found in patients with sickle cell anemia and occasionally in thalassemia major and in other severe anemias (Fig. 11).

### TEAR DROP AND PEAR FORMS

Erythrocytes with blunt or pointed filaments (Plate XIX G; Figs. 10 E; 11) are common in pernicious anemia but are not specific for this disease. Tear drop and pear forms are thought to be formed as a result of the fragmentation of the cytoplasm of abnormally formed nucleated red cells.

### MISCELLANEOUS FORMS

In addition to the abnormal shapes described above there are numerous other types of bizarre (Plate XIX H) and distorted cells, including "dumbbell," "pincered" and "triangular" forms. Broken off filaments of spinous processes of erythrocytes (schizocytes) may appear as tiny elongated or splinter-like structures or as fragments of varying size and shape.

## *Variations in Color (Anisochromia)*

Variation in color of erythrocytes is a function of the amounts of acidophilic and basophilic substance and the size and shape of the cells

### HYPOCHROMIC ERYTHROCYTE (ERYTHROCYTE WITH HYPOCHROMIA)

A hypochromic red cell is one in which there is a significant decrease in the density of the hemoglobin (red) color (Plate XX B). This decrease in the intensity of the staining may be due to a decrease in the concentration of hemoglobin or to an abnormal thinness of the cell or to both. In hypochromic cells the area of central achromia is enlarged. Cells that have a narrow rim of hemoglobin surrounding a large central pale area are known as "pessary cells" or "annulocytes."

Anemias characterized by erythrocytes with a significant decrease in the mean corpuscular hemoglobin and/or mean corpuscular hemoglobin concentration are designated as hypochromic anemias. Hypochromia and microcytosis are related phenomena.

### HYPERCHROMIC ERYTHROCYTE (ERYTHROCYTE WITH HYPERCHROMIA)

Hyperchromic red cells are cells which have a more intense hemoglobin (red) color than do normal cells (Plate XX C). The apparent increase in acidophilic substance is not due to an increase in the concentration of hemoglobin in a unit volume but to an increased thickness and volume of the macrocytic cell. There is more hemoglobin in the cell because the cell is larger than normal.

In certain of the macrocytic anemias the mean corpuscular hemoglobin may be increased, but the term "hyperchromic" as applied to anemias is not recommended, for the mean corpuscular hemoglobin concentration is seldom increased beyond the range of normal variation

## DIFFUSELY BASOPHILIC ERYTHROCYTE (ERYTHROCYTE WITH POLYCHROMASIA, POLYCHROMATOPHILIC ERYTHROCYTE)

Polychromatophilic erythrocytes are red cells with a bluish-red tinge due to an admixture of the colors of hemoglobin and of basophilic ribosenucleoprotein (Plate XX D). Diffusely basophilic cells are larger than normal and are interpreted as immature non-nucleated cells or proerythrocytes. The residual basophilia within the cells disappears as the cells age.

Diffusely basophilic cells are increased in the peripheral blood in those conditions in which there is an abnormal proliferation of erythrocytes in the bone marrow. Polychromasia is therefore a sign of active erythropoiesis or regeneration An absence of polychromatophilic cells and other immature cells in the presence of anemia would be a sign of hypoplasia or aplasia.

Cells which take a diffusely basophilic stain with Wright's would, if stained by a supravital stain before fixation, appear as reticulocytes

## STIPPLED CELL (ERYTHROCYTE WITH PUNCTATE BASOPHILIA, BASOPHILIC STIPPLING, BASOPHILIC AGGREGATION)

Stippled cells are red blood cells which contain multiple small dotlike structures or granules which take a basic or bluish stain with Wright's (Plate XX F). In contrast to the diffusely basophilic cells in which the basic ribosenucleoprotein is evenly distributed, the basophilic substance in stippled cells is aggregated or precipitated. The granules vary in size and shape. Some have threadlike attachments. They may be arranged in circles of varying size and simulate Cabot's rings. In erythrocytes in which there is coarse stippling the cells are often deformed, and there are areas deficient in hemoglobin and irregularities in hemoglobin distribution. Stippling is observed in nucleated red cells (Plate XXI B, F) and in diffusely basophilic cells (Plate XX F) as well as in cells with mature red cytoplasm

In blood smears in which there is an increased number of stippled cells there is usually an increase in the number of reticulocytes but there is poor correlation between the stippled cell count and the reticulocyte percentages.

In erythrocytes in which stippling is present there may be granules which give a positive test for iron (siderotic granules), but the majority of basophilic granules do not contain iron The stippled structures give a negative Feulgen reaction and therefore do not contain desoxyribosenucleoprotein.

On careful searching of ideally stained thin smears of normal individuals it is possible to find occasional erythrocytes with fine stippling of the cytoplasm, but coarse stippling is not seen in the smears of blood from normal individuals Stippled cells are found in many anemias and in the leukemias. Stippling is frequently observed in patients who are exposed to toxic drugs or heavy metals such as lead

In the lead industry the counting of the number of stippled cells is a standard procedure. One method is to count 50 fields of 50 cells each or a total of 2500 erythrocytes. Less than 10 stippled cells in 2500 erythrocytes is considered to be within acceptable limits, but a stippled cell count of above 10 is considered as significant and a sign of undue exposure to lead. The number of stippled cells is not a reliable index of lead intoxication.

## ERYTHROCYTE WITH SCHÜFFNER'S GRANULES (MALARIAL STIPPLING)

In erythrocytes infested with *Plasmodium vivax* (benign tertian) parasites, there are often multiple small, irregularly shaped red-staining particles or acidophilic granules. In erythrocytes containing parasites in the early ring stages the acidophilic granules are lightly stained and ill-defined, but tend to become more darkly stained and more prominent in the later maturation stages of the parasite and in gametocytes The granules are fairly uniform in size and evenly distributed Schuffner's granules differ from the granules in stippled cells in that the Schuffner's granules are predominantly acidophilic rather than basophilic.

## SIDEROCYTE (PAPPENHEIMER BODY CELL)

Siderocytes are erythrocytes which contain small particles of nonhemoglobin iron (Fig 15). In the Wright's stain these bodies, often called "Pappenheimer bodies," have a dark purple-blue to black color. When treated with potassium ferrocyanide and hydrochloric acid, these granules appear greenish-blue to black. In the unstained preparations the siderotic granules are refractile and colorless bodies. They vary in size from barely visible granules to objects that may be 1 to 2 *micra* in diameter. The majority of granules are round but they may be elongated or irregular in shape They tend to be unevenly distributed and often appear at the periphery of the cell. Single iron-positive granules may be present in a given red blood cell, but as a rule there are several granules, and in rare instances there may be 20 or more siderotic granules in a single erythrocyte The granules frequently appear in pairs or in small groups (Fig. 15).

Siderotic granules as a class are smaller and less spherical than Howell-Jolly bodies. They are larger and more intensely stained and less evenly distributed than the granules of "stippled cells." It is often impossible, without the use of the special iron stain, to look at a given granule and to say with certainty that it is a siderotic granule. Often in regenerative anemias siderotic granules may be present in cells which also contain Howell-Jolly bodies and basophilia of a granular (punctate) type In conditions in which siderocytes are demonstrable in the peripheral blood the reticulocyte count is usually significantly increased, but there is poor correlation between the siderocyte count and the reticulocyte count

Stainable ferric iron particles (siderotic granules) appear in varying numbers in the nucleated red cells in bone marrow smears of normal individuals and are thought to represent free or nonhemoglobin iron particles within the cell, previous to its synthesis into the hemoglobin molecule. Nucleated red cells containing siderotic granules are called "sideroblasts" In conditions in which there is a deficiency in iron, the siderotic granules in the nucleated red cells are reduced or absent The number of siderotic granules in nucleated red cells is significantly increased in megaloblastic anemias, thalassemia, eryth-

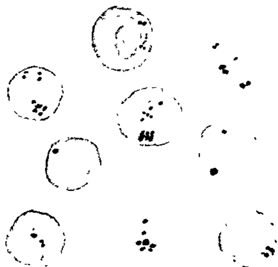


*Figure 15.* Siderotic granules in erythrocytes Prussian blue stain

remic myelosis and in other conditions in which there is faulty hemoglobin synthesis.

Siderocytes are not demonstrable in the peripheral blood of normal individuals They are frequently seen in patients with sickle cell anemia, thalassemia and other hereditary and acquired types of hemolytic anemia. Siderotic granules are also observed in the erythrocytes of patients exposed to lead, other heavy metals and cytotoxic drugs. They are conspicuous in hemolytic anemias following splenectomy It is thought that the erythrocytes containing iron are phagocytized readily by the spleen and that following splenectomy the defectively formed and abnormal cells containing iron granules survive longer in the circulation.

## RETICULOCYTE (RETICULATED ERYTHROCYTE)

Reticulocytes are immature erythrocytes which when stained with brilliant cresyl blue or other supravital dye reveal a reticulum (netlike) or granulofilamentous structure. The linear and granular material is not visible in unstained preparations nor in dried smears stained by the Wright's method In order to see the reticulum in Wright's stained smears, the cells must first be exposed to the supravital dye which penetrates the membrane of the living cell and stains the reticular structure The cells may then be fixed and counterstained (Plate XX E, Fig. 16) (For methods see Chap IX )

The reticulum is most prominent in the early nucleated red cells where it tends to form intertwined granular fibrils or heavy aggregates in the cytoplasm In the older nucleated red cells and in the non-nucleated red cells soon after the nucleus is dissolved or extruded, the reticular material becomes progressively less prominent and less filamentous and more granular It is estimated that the reticulum remains demonstrable in the cytoplasm of erythrocytes for two to four days after the cell gets into the circulating blood In the final stage the reticulum consists of a few fine granules

Reticulocytes as a class are larger than nonreticulated and senile cells. They are usually thicker and more spherical than are nonreticulated cells. There is evidence that they are more resistant than are older erythrocytes to hemolytic processes.

Under normal conditions there are 1 to 20 reticulocytes per 1000 erythrocytes, with an average of approximately 10 per 1000 erythrocytes. In conditions in which there is increased regeneration and delivery of erythrocytes into the circulation, the reticulocytes are increased, often above 10 per cent. In rare instances in which there is rapid cell destruction and compensatory formation of new cells, 75 to 95 per cent of the red cells may be in the reticulocyte stage. Increases in reticulocytes are common following blood loss, in congenital and acquired hemolytic anemias and in the recovery phase after marrow aplasia. A low or decreased reticulocyte count in the presence of anemia is indicative of inability of the bone marrow to respond to an hypoxic stimulus.

In conditions such as pernicious or other macrocytic anemias in which there is a defi-



*Figure 16* Reticulocytes with granulofilamentous substances Brilliant cresyl blue moist preparation.

ciency of folic acid and/or vitamin $B_{12}$, the marrow is hypercellular but the cells are immature and there is faulty release of cells from the fixed tissue depot into the flowing blood. The number of reticulocytes delivered to the circulating blood is normal or only slightly increased. When the deficient factor is supplied in adequate amounts, millions of immature cells which lack specific growth factors simultaneously mature. The blast cells develop into prorubricytes and later into rubricytes and metarubricytes Finally the stage of maturation is reached in which numerous non-nucleated erythrocytes containing granulofilamentous substance are liberated in great numbers into the peripheral blood. The increase in the percentage of reticulocytes begins immediately after the injection of effective liver extract or vitamin $B_{12}$ and reaches its peak in four to six days. The shower of reticulocytes lasts for six to ten days after which the normal division, maturation and delivery of cells is established and the percentage of reticulocytes returns to normal. A similar temporary increase in the percentage of reticulocytes follows the giving of iron to patients who have anemia of an iron deficiency type and the giving of thyroid extract to patients with anemia associated with myxedema.

The number of cells having demonstrable reticular structure as revealed by supravital methods corresponds with the summation of all the immature cells in the Wright's stain which contain nuclei or nuclear fragments and/or which contain basophilic cytoplasmic substance in a diffuse or punctate (stippled) form.* There is usually a close correlation between the number of diffusely basophilic cells and the number of reticulocytes. In lead exposure in which there is an increase in stippled cells there is an increase in reticulocytes, but the number of stippled cells is poorly correlated with the reticulocyte count. In conditions in which there are numerous stippled cells, the correlation is between the sum of diffusely basophilic cells and stippled cells.

## CABOT'S RING CELL

Cabot first described delicate annular structures in erythrocytes (J. Med Research 9:15, 1903). The ring takes a purple red to red color in Wright's stain (Plate XXI H, L). The Cabot's ring varies in size from that of a small circle several micra in diameter to large structures having a peripheral arrangement. On casual examination the ring appears to be a continuous line, but on careful examination it is observed to be composed of small acidophilic granules which are arranged in a lineal pattern. In the majority of Cabot's rings there is one continuous circle or loop, but in others there are double or multiple concentric lines. Variants include "figure of eight" forms and intertwined multiple loops (Fig. 17) In some of the cells the granules may be arranged in a narrow line in one portion and diffusely or unevenly scattered in another portion of the arc Some of the rings may have scroll-like extensions from their margins (Plate XXI H, L; Fig. 17)

The cells containing Cabot's rings vary in size and are often unevenly stained and ab-

* The placement of the "reticulocyte" in the maturation sequence between the metarubricyte and the mature red cell or normocyte is not recommended, for all stages of nucleated red cells as well as erythrocytes, immediately after losing their nuclei have demonstrable granulofilamentous substance in their cytoplasm when stained by supravital techniques It is confusing to portray all of the other cells as they appear with ordinary Wright's stain and picture one cell in the sequence as it appears when stained by another method In the normal sequence of maturation in Wright's stained smears the cell following the metarubricyte should be a diffusely basophilic erythrocyte or proerythrocyte and not a "reticulocyte" (Plate VIII)

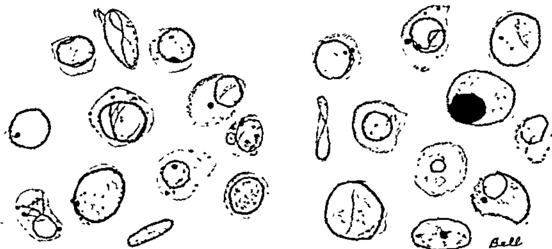



*Figure 17.* Nucleated and non-nucleated erythrocytes with Cabot's rings and annular cytoplasmic structures.

normally shaped. It is not uncommon for the Cabot's ring cell to contain basophilic granules and nuclear fragments. Occasionally the rings are present in nucleated red cells. They may encircle the nucleus or be independent of the nucleus Ringlike structures are sometimes demonstrable as annular bodies outside of the erythrocytes, indicating that they are more resistant than the erythrocyte and remain as a morphologic entity after the red cell is destroyed

Cabot's ring cells are rare. They are not found consistently in any disease condition. They may be present in the smears of a given patient for a day or several days and disappear later on They are most frequently seen in conditions in which there is a severe alteration in erythrogenesis as in untreated pernicious anemia, lead poisoning, sickle cell anemia, leukemia, thalassemia and erythremic myelosis Cabot's rings are manifestations of immature cells rather than senile forms, but their exact nature is not known.

## Nucleated Red Cells

### NUCLEATED RED CELLS IN THE PERIPHERAL BLOOD

Nucleated red cells are present in the circulating blood of newborn infants but are not present in stained blood smears of normal individuals after the first few days of life. The presence of nucleated red cells (erythroblasts) is indicative of active erythrocytic proliferation These cells may be present in the circulating blood in acute blood loss anemia, pernicious anemia, hereditary and hemolytic anemia, erythremic myelosis, leukemia, recovery from an aplastic state, chemical poisoning, metastatic malignancy and many other conditions. As a rule the erythrocytes with nuclei in the peripheral blood are in the more mature or metarubricyte stage of maturation, but less mature cells such as rubricytes, prorubricytes and in rare instances rubriblasts may be seen

## MITOTIC FIGURES

Mitotic figures in nucleated cells of the erythrocytic series are present in small numbers in smears of bone marrow from normal individuals. Mitotic figures in excess of 10 per 1000 nucleated erythrocytes are indicative of abnormal proliferative activity.

## POLYPLOID NUCLEATED RED CELLS (GIANT ERYTHROBLASTS)

The separation of the nucleus without a corresponding division of the cytoplasm (karyokinesis without cytokinesis) and pluripolar mitoses occur in pathologic conditions in which there is stimulated erythropoiesis, such as pernicious anemia (Plate XXI B), hemolytic anemia and erythremic myelosis.

The production of the giant nucleated red cells (giant erythroblasts) is not necessarily a new line of cell development which will continue to undergo abnormal mitoses and constitute an irreversible and malignant family of cells. It is apparent that some of the plurinuclear cells will degenerate without reproducing new cells. Others will later divide into as many daughter cells as there are separate nuclei The daughter cells will later divide normally The multiple nuclei do not necessarily interfere with the maturation of the cytoplasm, for one may find plurinuclear cells with all stages of cytoplasmic morphology from cells which are deeply basophilic and contain little hemoglobin to cells which are orthochromatic and contain little or no basophilic substance.

## NUCLEAR FRAGMENTS

In pathologic states one frequently sees nucleated red cells with multiple lobulations (Plate XXI B) and aberrant nuclear masses (Plate XXI D) which may be free in the cytoplasm or connected by fine filaments These structures probably represent the end stages of pluripolar mitoses or defects in nuclear cleavage, with deformity in nuclear pattern and nuclear shape. It is probable that some of the multiple nuclear fragments are produced during the process of nuclear dissolution and fragmentation. Plurinuclear erythrocytes in the peripheral blood are indicative of abnormal erythropoiesis but are not specific for any one disease and are not necessarily associated with malignant conditions.

## HOWELL-JOLLY BODIES

Howell-Jolly bodies are spherical, structureless masses in the cytoplasm of erythrocytes, 1 to 2 micra in diameter (Plate XXI H, K). They are visible in the phase contrast microscope and are Feulgen-positive. They are usually single but may be multiple. They are occasionally seen in nucleated erythrocytes and in cells which contain Cabot's rings and basophilic substances of the diffuse or punctate type. Howell-Jolly bodies are considered as the end stages of nuclear fragmentation. Another explanation for these bodies is that they are adherent pyknotic chromosomes which "strayed from the flock" during the process of mitosis and were not included within the nuclear membrane during the reorganization of the chromatin

Howell-Jolly bodies are to be differentiated from bacteria and fungi which underlie or overlie the erythrocyte. Howell-Jolly bodies are within the cytoplasm and stand out sharply when the erythrocyte is in focus, whereas structures outside of the cell are best seen when the erythrocyte is slightly out of focus. Artefacts have a tendency to form highlights and shadows and to have a light area around them, caused in part by the thinning of the indented erythrocyte and in part by the refraction of light rays at the margins of the artefact, producing a halo of brightness Howell-Jolly bodies are to be differentiated from platelets (thrombocytes) superimposed on the erythrocyte by the fact that thrombocytes have granules and are surrounded by a pale zone (Plate XXI J).

Howell-Jolly bodies are demonstrable in many regenerative anemias They are particularly prominent in sickle cell anemia and in patients following splenectomy

## MEGALOBLASTIC NUCLEATED RED CELLS (MACROCYTIC ERYTHROBLASTS, NUCLEATED RED CELLS OF THE PERNICIOUS ANEMIA TYPE)

Megaloblastic nucleated red cells are defined as abnormally large and dysplastic nucleated erythrocytes which are found in the bone marrow of patients with pernicious anemia and related macrocytic anemias (Table 7). In severe untreated pernicious anemia these cells constitute a distinctive line or family of megaloblastic (macrocytic) cells which are morphologically different from the normal (normoblastic) cells and from the hypochromic microcytic cells (microblastic cells) (Plate VIII).

The primitive cell of the megaloblastic or macrocytic sequence of nucleated red cells is called a megaloblast (rubriblast of the *pernicious anemia type*). This cell is larger than the rubriblasts found in marrow smears of normal individuals. The cytoplasm is dark with varying amounts of red and blue color. The color of the cytoplasm resembles that of plasmocytes The cytoplasmic area next to the nucleus is stained less intensely than the margins of the cell. Contained within the cytoplasm are numerous chromophobic mitochondria which give to the cytoplasm an unevenly stained appearance (Plate VIII, Fig. 18). The nuclei of megaloblasts are large, round, and have a finely reticulate structure. Nucleoli are usually demonstrable.

In the later stages of maturation of the megaloblastic series of erythrocytes the cells are abnormally large (Fig 18) The synthesis of hemoglobin in the macrocytic cells proceeds at a more rapid rate than the development of mature nuclear characteristics. This dissociation in the maturation of nucleus and cytoplasm leads to the production of many cells which have an immature chromatin structure and a full component of hemoglobin In some cells there are localized islands of acidophilic substance (hemoglobin) surrounded by bluish cytoplasm, giving to the cells a mottled appearance. In other cells there are chromophobic areas (Plate XXI B). The macrocytic and dysplastic character of the marrow cells is not confined to the erythrocytic elements, but also involves the granulocytes (Figs. 18, 20, 21) and megakaryocytes. These cells, like the nucleated red cells, are macrocytic. Many of the neutrophilic cells are abnormally lobulated and ultimately mature into macrocytic hypersegmented cells, called "P.A. polys."

It has been traditional to classify nucleated red cells into "normoblastic" and "megaloblastic" categories, the assumption being that there are two natural or normal lines of red cell development. This method of tallying cells is rational when one is dealing with a patient who has classical pernicious anemia. It is impractical as a routine procedure to enumerate the megaloblastic cells, for most of the marrow smears are from patients with conditions other than pernicious anemia. The majority of patients with pernicious anemia have received treatment with specific substances before the marrow is examined. This leads to the formation of intermediate or transitional forms which cannot, except by "tossing the coin," be placed in the megaloblastic or normoblastic column. Iron deficiency anemia is much more common than pernicious anemia and is often combined with deficiencies of vitamin $B_{12}$ and/or folic acid. Many abnormal nucleated erythrocytes are neither megaloblastic nor normoblastic but are microblastic.

There is no valid reason for maintaining a special terminology for macrocytic nucleated red cells in the performance of routine differential counts on marrow. The pathologic cells found in pernicious anemia and related diseases should be called rubriblasts (proerythroblasts), prorubricytes (basophilic or early erythroblasts), rubricytes (polychromatophilic or intermediate erythroblasts) and metarubricytes (orthochromatic or late erythroblasts) (Plate VIII). A description of the

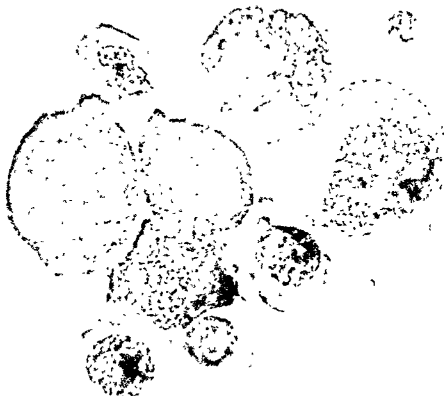


*Figure 18* Photomicrograph Bone marrow smear Pernicious anemia Right upper cell is hyperlobulated band neutrophil Largest cell to right is macrocytic myelocyte with vacuole and "dawn of neutrophilia." Other cells are varying stages of macrocytic nucleated red cells or megaloblasts (erythroblasts or rubriblasts of *pernicious anemia type*)

size and nuclear characteristics should be added (see Chap. I).

The phrase *"pernicious anemia type"* has the great advantage over *megaloblastic* in that it can be applied to the cells of the granulocytic as well as to the erythrocytic series (Am J. Clin Path, *19:* 56–60, 1949).

### MICROCYTIC NUCLEATED RED CELLS (MICROBLASTS)

Although volumes have been written about the megaloblasts and special names given for the various stages in the developmental stages in the macrocytic sequence, little attention has been paid to the much more common and equally as important but less conspicuous family of cells, the microblasts or the abnormally small nucleated red cells. Fortunately no special names have been applied to these cells They therefore are classified as rubriblasts, prorubricytes, rubricytes and metarubricytes, followed by a description of the observed abnormalities (Plate VIII).

Microcytic nucleated cells are increased in the bone marrow in conditions in which there is iron deficiency, as in blood loss and in nutritional deficiency diseases, or defects in the synthesis of iron, as in infectious diseases and in hereditary thalassemia. Microblasts, in addition to being small, are deficient in hemoglobin and have a bluer cytoplasm than do normal nucleated cells They are frequently misshapen and have pointed cytoplasmic tags (Plate VIII) The cytoplasmic development lags behind the maturation of the nucleus which usually has a condensed chromatin structure and is darkly stained

Microblasts, with their pyknotic nuclei, narrow rim of bluish cytoplasm and pointed filaments, closely resemble small lymphocytes. In thick portions of the smears and in poorly stained preparations it is impossible to differentiate some of the microblasts from lymphocytes except by association.

## Other Abnormalities

### ROULEAUX FORMATION

In thick moist preparations and in thick blood smears of erythrocytes the cells stack up like coins (rouleaux formation). In many pathologic conditions the tendency to gather in rouleaux formation is exaggerated and it may be difficult to make thin smears in which the cells are well separated. The aggregation of cells is striking in plasma cell myeloma and many other conditions in which there is an increase in fibrinogen and globulin and a relative decrease in albumin. In conditions characterized by a marked rouleaux formation the sedimentation rate is increased and the background color of the blood smear is darker than normal.

Rouleaux formation is to be differentiated from agglutination. Agglutinated cells are clumped without orientation The margins of the cells in the agglomerated mass have an indistinct contour. Slight dilution does not cause them to break up Gentle agitation of the blood favors the exaggeration of the process Cells in rouleaux formation, on the other hand, are stacked like coins in orderly columns, disk to disk. The cell membranes are intact. The stickiness of the cells and the attachment to each other becomes significantly less marked on slight dilution The agitation of the suspension causes temporary dispersal of the units



*Figure 19.* Photomicrograph Crystalline hemoglobin structures within and outside of erythrocytes. Peripheral blood smear. Homozygous hemoglobin C disease. (Diggs et al ; Blood *9*(12) 1172, 1954.)

## ERYTHROCYTE WITH TETRAGONAL HEMOGLOBIN CRYSTALS

Erythrocytes containing dark *elongated* structures having a hexagonal shape have been reported in patients with homozygous hemoglobin C disease (Fig 19) Exaggeration in the number of crystals in moist preparations of blood occurs following simple partial drying procedures.

## *SEMILUNAR BODIES* (CRESCENT BODIES)

*Semilunar bodies are long, pale, narrow* bluish pink nongranular structures which have a crescent moon shape (Plate XX G). They are thought to be the degenerative remains of erythrocytes or smudges of erythrocyte stroma They may be found in smears of normal blood but are most often seen in hemolytic anemias and in malaria. They have no diagnostic significance.

# Chapter V

# PATHOLOGIC LEUKOCYTES

## Neutrophils

### HYPERSEGMENTED MACRONEUTROPHIL (HYPERFILAMENTED MACRONEUTROPHIL, "P.A. POLY")

Hypersegmented macroneutrophils are abnormally large neutrophils with six or more distinct lobes (Plate XXII G; Fig 20) Macrocytic neutrophils with an increase in nuclear segmentation are called "P.A. polys" because they are constant findings in the blood of patients with untreated pernicious anemia and related macrocytic anemias (Table 7). These abnormal leukocytes appear early in deficiencies of vitamin $B_{12}$ and folic acid and are the last to disappear after specific therapy. They may be the only definitive sign of hematologic abnormality in subacute combined degeneration of the central nervous system.

The hypersegmented cells ("P.A. polys") in the peripheral blood are preceded by cells in the bone marrow which are likewise abnormally large and hyperlobulated. The metamyelocytes and band stages of the macrocytic family of cells have deep nuclear grooves and folds and prominent nuclear projections (Figs 18, 20, 21)

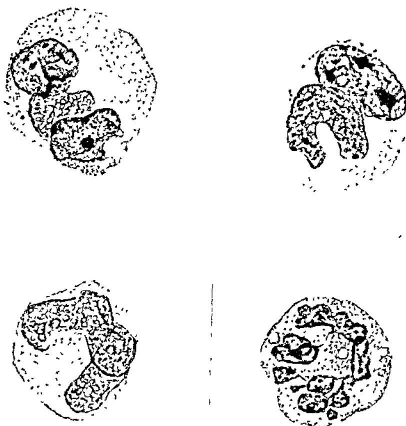


*Figure 20* Hypersegmented macroneutrophils from bone marrow smear of patient with untreated pernicious anemia

Hypersegmented macroneutrophils are to be differentiated from giant polyploid neutrophils (Plates XXII E, XXIII I) Both types of neutrophils are large and have multiple nuclei. The lobes of the "P.A poly" (hypersegmented neutrophil) are connected by filaments, whereas one or more lobes of the multinucleated polyploid cell are separate.

Metamyelocytes and band forms of the "pernicious anemia" type (Figs 18, 20, 21) morphologically resemble monocytes Both cell types are large and have multiple nuclear folds and indentations. Degenerative vacuoles in the cytoplasm of macroneutrophils simulate the chromophobic digestive vacuoles of the monocyte (Fig 21) The differentiation is based for the most part upon the association with other cells and the finding of transitional forms One should be able to find unmistakable hypersegmented neutrophils as running mates for hyperlobulated macrometamyelocytes and band forms. Monocytes are relatively rare in bone marrow smears except in those conditions in which there is malignancy involving the histiocytic-monocytic system or in which there is a significant increase in monocytes in the peripheral blood A questionable granular cell with an indented nucleus in the bone marrow is more likely to be a neutrophilic metamyelocyte or band form than a monocyte

Although giant neutrophils with hyperlobulation are most frequently seen in pernicious anemia, *such cells are not specific for*

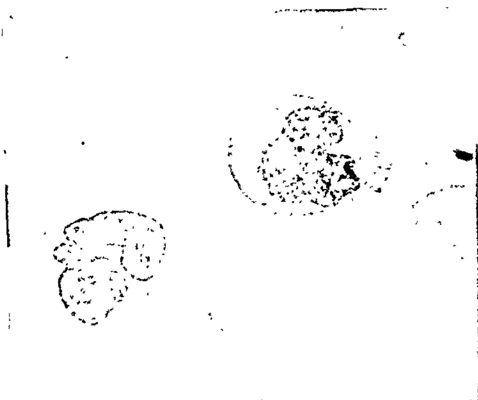


*Figure 21* Photomicrograph of hyperlobulated macroneutrophils with vacuoles. Note full hemoglobin content of erythrocytes

this disease They are also found in myeloproliferative diseases such as chronic myelocytic leukemia, myelosis and polycythemia Multilobulated and abnormally large neutrophils develop in patients with leukemia who are treated with "anti-folic" drugs, such as Aminopterin, which compete with folic acid and are inadequate substitutes for the essential vitamins in the synthesis of nuclear proteins.

## HYPOSEGMENTED NEUTROPHIL

Hyposegmented neutrophils are neutrophilic granulocytes which have the cytoplasmic characteristics of mature cells and the nuclear shapes of immature cells The dissociation between the shape of the nucleus and the cytoplasmic features of the cells is the opposite of that seen in hypersegmented cells The nonlobulated shape of the nucleus may be due to a defect in maturation of the nucleus or a manifestation of degeneration

The inhibition of nuclear lobulation may be an inherited characteristic, known as the *Pelger-Huet anomaly.* In this condition there is a decrease in segmented forms There is an increase in band forms and cells with indented and round nuclei The nuclei have a coarse and lumpy chromatin structure The band forms are short and thick. Many of the two-lobed neutrophils have a pince-nez shape. The maturation defect is not limited to the neutrophils, for the eosinophils, basophils and monocytes are similarly involved. The Pelger-Huet anomaly is not associated with toxic granules or vacuoles.

Hyposegmentation of the nucleus is noted in severe infections, in toxic states, in poisoning by cytotoxic substances and in malignancies of the myelocytic system (Plate XXII F). The nuclei of such cells are often pyknotic. Associated signs of abnormality include decreased motility, loss of ability to phagocytize, toxic granules and vacuoles (Plate XXII

A, B). The presence of nonlobular cells with pyknotic nuclei is spoken of as "a degenerative shift to the left" in contrast to the more usual abnormality, "regenerative left shift," in which there is an increase in the number of band cells and other immature forms without signs of degeneration.

In body fluids such as cerebrospinal fluid, urine, fluids from serous cavities and exudates, the nuclei of segmented neutrophils undergo degenerative changes. The filaments connecting the lobes of some of the segmented cells disappear, the lobes become spherical and the chromatin becomes condensed (Plate XXII D). In some instances the lobes fuse into a single spherule while in others there is fragmentation of the nucleus, producing multiple dark-staining masses of different sizes. In the performance of differential counts on body fluids and exudates it is obvious that the shape of the nucleus and the chromatin structure are unreliable as criteria of identification.

Degenerative nuclear changes similar to those observed in body fluids and exudates rapidly take place in specimens of peripheral blood or bone marrow after removal from the body (Plate XXII D) Smears of the buffy coat of defibrinated blood or blood to which heparin or other anticoagulants have been added often reveal a reversion of lobulated nuclei to a spherical and pyknotic form in some of the cells Similar nuclear degenerative changes are observed in smears of blood removed at autopsy. Cytolysis and nucleolysis, particularly of neutrophils, takes place so rapidly after death that it is difficult to perform accurate differential counts on blood and bone marrow smears of autopsied cases

## TOXIC GRANULES

The granules of a normal neutrophil when stained properly by the Wright's method are faint pink or lavender and are inconspicuous In certain patients with poisoning, severe infections, agranulocytosis and myeloproliferative diseases, the granules in the neutrophils may be prominent and have a dark blue color. Such granules are known as "toxic granules" (Plate XXII A, C).

The overstaining of the smear or the use of a too alkaline buffer solution as diluting fluid for the Wright's stain will cause the granules of normal neutrophils to be prominent and dark. One therefore must be sure that the stain is satisfactory before "toxic granules" are reported

## *VACUOLES*

The so-called vacuoles in white cells are spherical unstained areas of various sizes (Plate XXII B; Figs. 20, 21). They may occur in the cytoplasm and/or in the nucleus. Vacuoles develop rapidly in neutrophils after removal from the circulating blood and are common in smears of fluids from serous cavities. Vacuoles are infrequent in the neutrophils of peripheral blood and bone marrow of normal individuals provided the smears are made immediately after collection. Their presence in such smears is most common in severe infections, chemical poisoning, cachectic states and leukemias (Fig. 29). Vacuoles in the cytoplasm and nucleus are often accompanied by toxic granules, pyknotic nuclei and irregularities in cytoplasmic staining.

## DOHLE'S INCLUSION BODIES

In infectious diseases such as septicemia, scarlet fever, measles and pneumonia, neutrophilic cells may be found which have small localized areas of bluish cytoplasm. Such structures are interpreted as the remains of an earlier basophilic material and, therefore, are manifestations of defective maturation.

## AUER BODIES (AUER RODS)

Auer bodies are small, elongated, rod, lanceolate or splinter-like forms which are sometimes seen in the cytoplasm of immature leukocytes. These cytoplasmic structures are thought to be crystalline forms of nucleopro-

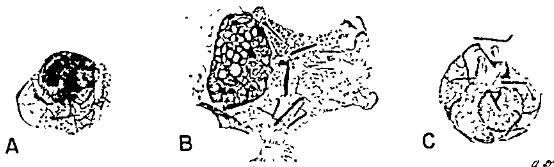



*Figure 22* India ink drawing. Auer rods *A*, Myelocyte. *B*, Granular histiocyte *C*, Monocytoid cell From the bone marrow smear of a patient diagnosed as myelocytic-monocytic-histiocytic leukemia. (Courtesy of Dr. Rosina Vincenzi, Atlanta, Georgia )

tein. There are usually one (Plate XXIII C) or several Auer rods in a given leukocyte (Fig. 22) Multiple crystals may be present (Plate XXIX D, Figs. 22, 33). The Auer bodies are red to purple in Wright's stained smears. The majority of cells containing Auer bodies are undifferentiated cells with no granules (Plate XXIII C) or a few nonspecific basophilic granules In rare instances Auer rods are demonstrable in neutrophilic and eosinophilic myelocytes, granular histiocytes (Ferrata cells) and monocytes (Fig 22). Elongated crystal-like structures, similar to those found in the cells of granulocytic series, are demonstrable in a small percentage of patients with plasma cell myeloma (Fig. 33, Plate XXIX D)

The presence of cytoplasmic inclusions of the Auer rod type in a patient with immature cells in the peripheral blood or an increase in immature cells in the bone marrow is presumptive evidence of leukemia These bodies are most frequently found in myelocytic and monocytic-histiocytic types of leukemia and have never been seen by the authors in lymphocytic leukemia or in malignancies involving the erythrocytic or megakaryocytic series Their presence in plasmocytes has never been observed in conditions other than plasma cell myeloma

## GRANULOCYTES IN GRANULOCYTIC LEUKEMIA

In the early stages of chronic granulocytic leukemia there is an increase in granulocytes having an essentially normal morphology (Plate II, Fig 23). There is a slight relative and absolute increase in immature forms, the majority of which are neutrophilic bands and metamyelocytes In the later phases of the disease the proportion of malignant cells increases, and the cells become more immature and atypical. In acute myelocytic leukemia and in the end stages of chronic myelocytic leukemia the majority of cells are myeloblasts

One of the common abnormalities in myelocytic leukemia is asynchronism in the nuclear shape and cytoplasm This asynchronism may be manifested by immature cells with indented nuclei or cells with mature cytoplasm and faint pink granules having a round nucleus. Granules may be abnormally prominent in some cells (Plate XXIII E) and defective in others. Additional forms of atypical cells are large cells which are morphologically borderline between granular histiocytes (tissue neutrophils) and progranulocytes (Plate XXIII F, G, H), atypical megakaryocytes (Plate XXIII B), giant polyploid cells, abnormal mitotic figures, immature cells with

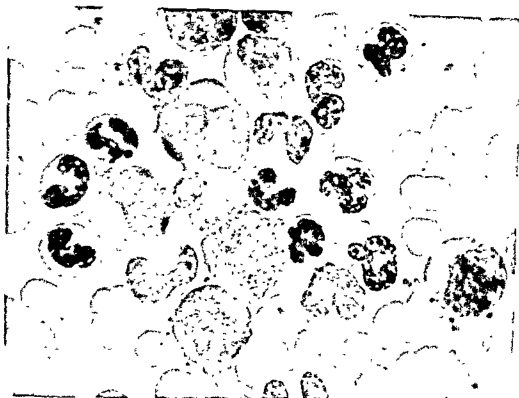


*Figure 23.* Photomicrograph. Peripheral blood Chronic granulocytic (myelocytic) leukemia Pleomorphism Platelets adequate

Auer rods (Fig. 22) and cells with vacuoles in the cytoplasm and nucleus

In performing a differential count of leukocytes in myelocytic leukemia one arbitrarily has to place many cells in a given category on the basis of combined morphologic evidence, for the criteria of normal cell morphology cannot be applied rigidly

In the majority of smears from patients with chronic myelocytic leukemia in the intermediate stages there is an absolute increase in eosinophils and basophils. Small numbers of typical granular histiocytes, monocytes, plasmocytes, lymphocytes, nucleated red cells and occasional megakaryocytes or megakaryocyte fragments are also present.

The cells of the granulocytic series give a strong peroxidase reaction (Chap IX) in *contrast to lymphocytes, erythrocytes, and* plasmocytes which give a negative reaction In conditions in which there are a large number of atypical immature and intermediate forms the peroxidase stain is of value in identifying questionable cells and in differentiating granulocytes from nongranulocytes (Plate XXIV) Myeloblasts give a negative reaction. Monocytic cells usually have a few peroxidase-positive granules.

## Eosinophils and Basophils

Eosinophils and basophils are subject to the same degenerative nuclear and cytoplasmic changes, abnormalities in mitosis and asynchronism in the maturation of nucleus and cytoplasm as are the cells of the neutrophilic series described above Eosinophils and basophils constitute such a small percentage of the leukocytes in most diseases that little useful clinical information is derived from a study of the morphologic variants

In eosinophilic and basophilic leukemias which are considered to be variants of granulocytic leukemia, the majority of cells in the peripheral blood are mature cells. As the

disease progresses, the highly differentiated cells are replaced by myeloblasts and progranulocytes without the appearance of large numbers of eosinophilic and basophilic myelocytes and metamyelocytes.

## Lymphocytes

### LYMPHOCYTES IN INFECTIOUS MONONUCLEOSIS

In infectious mononucleosis there is a pleomorphic cellular pattern with numerous atypical cells (Plate XXV). The predominant cells are the lymphocytes which usually constitute 60 per cent or more of the leukocytes. The lymphocytes vary greatly in size with a tendency toward an increase in large forms (Fig 24). Many of the lymphocytes have scalloped edges and are deeply indented by adjacent erythrocytes (Plate XXV G, Fig. 24). Azurophilic granules are prominent in some of the lymphocytes (Plate XXV H). In addition there are many atypical cells which include:

1. Plasma-like lymphocytes, with dark blue cytoplasm and round, often eccentric nuclei (Plate XXV A, B, C).

2. Immature cells with fine chromatin structure and nucleoli simulating "blasts" (Plate XXV A).

3. Lymphocytes with a foamy appearance and vacuoles in the cytoplasm and nucleus (Plate XXV E; Fig. 24).

4. Large lymphocytes with uneven staining cytoplasm Some of the cells have a ruffled or pleated appearance which has led to the

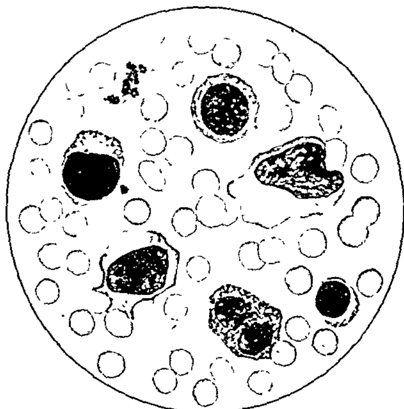


*Figure 24* Peripheral blood, infectious mononucleosis Variety of cell forms (Water color drawing by Doris McGowan )



*Figure 25* Photomicrograph. Peripheral blood smear Wright's stain Chronic lymphocytic leukemia Lymphocytosis with lumpy nuclear chromatin and narrow rim of cytoplasm. One smudged cell. Thrombocytes decreased

designation of this cell as "the ballerina skirt" cell (Plate XXV D).

5. Atypical large lymphocytes with indented nuclei and opaque, ground glass and bluish-grey cytoplasm which resemble monocytes and cannot on morphologic grounds be differentiated with surety from them (Plate XXV F).

The combinations of cell variants of the types described above are characteristic of infectious mononucleosis, but similar cells may be found in small numbers or for short periods of time in other virus diseases including virus hepatitis, infectious choriomeningitis, the acute exanthemata of childhood, virus pneumonia and influenza.

In lymphocytic and monocytic leukemia one may see cells which are morphologically similar to those observed in infectious mononucleosis, but in leukemia there is a monotonous repetition of the same type of abnormal cell (Figs. 25, 27) in field after field, with a paucity of other cell types. In infectious mononucleosis there is marked pleomorphism, with normal cells intermingled with pathologic forms and with few fields alike (Fig. 24) In infectious mononucleosis the thrombocytes are normal, and nucleated red cells and typical blast forms are absent or extremely uncommon In leukemia, on the other hand, there is a thrombocytopenia, and nucleated red cells and "blasts" are frequent or may be found readily on searching.

## LYMPHOCYTES IN LYMPHOCYTIC LEUKEMIA

The majority of lymphocytes in chronic lymphocytic leukemia are small, with a narrow rim of cytoplasm and a mature nuclear structure (Fig 25). In the more acute lym-

phocytic leukemias and in the terminal stages of chronic lymphocytic leukemia there is greater variation in the size and structure of the cells and an increased number of large lymphocytes, prolymphocytes and lymphoblasts (Plate XXVI A, B, C). Variants include mitotic figures, multiple nuclei (Plate XXVI E), cells with nuclear clefts and cleavage lines (Plate XXVI G, H; Fig. 26), herniation of nuclear material through the nuclear membrane and fragments of nuclear material free in the cytoplasm (Plate XXVI I).

The cytoplasm of the majority of cells in lymphocytic leukemia takes a nonspecific blue and rather homogeneous stain. Azurophilic granules which may be prominent in normal lymphocytes (Plate IV I, K) and in infectious mononucleosis (Plate XXV H) are rare in lymphocytic leukemia There are occasional lymphocytes with a fine reticular cytoplasmic structure, some of which have a laminated pattern (Plate XXVI C, D). Abnormal amounts of acidophilic hyaline material, simulating plasmocytes, may be demonstrable (Plate XXVI F).

In lymphocytic leukemia spindle cells and cells with frayed and hairlike cytoplasmic projections are common Smudges are numerous and often outnumber the cells which have intact nucleus and cytoplasm (Plate XXVI J, Fig. 25). In the early stages of chronic lymphocytic leukemia the thrombocytes are essentally normal and nucleated red cells are not demonstrable, but in acute leukemias and in later stages of chronic lymphocytic leukemia immature cells of the erythrocytic series appear in the circulating blood in small numbers and thrombocytes are significantly decreased.



*Figure 26* Cleft nuclei in lymphocytes Bone marrow smear, lymphocytic leukemia

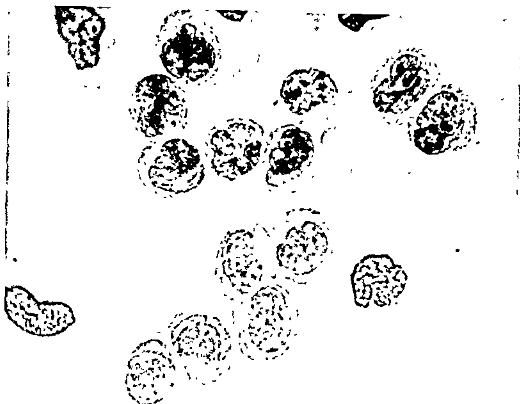


*Figure 27* Photomicrograph Peripheral blood. Monocytic leukemia, Schilling type Indented and folded nuclei Granular cytoplasm Smudged leukocytes Thrombocytopenia

One of the common errors in the diagnosis of leukemia is the improper identification of undifferentiated cells of the myelocytic or monocytic series as lymphocytes Thin smears, critical stain, a bright light and a good microscope are essential in visualizing the nuclear structure and the cytoplasmic granulation. In case of doubt the diagnosis should be "stem cell leukemia" rather than lymphocytic leukemia

## Monocytes

### PHAGOCYTIC MONOCYTE

One of the characteristics of monocytes is the capacity to phagocytize particulate matter Objects commonly phagocytized include red blood cells (Plate XXVII I) and red cell fragments, nuclear particles, and pigment. They may contain etiologic agents, such as bacteria, rickettsiae, fungi or protozoa. Large vacuolated monocytes which may contain phagocytized objects may be conspicuous in smears of patients with subacute bacterial endocarditis, typhoid fever and other types of septicemia.

### MONOCYTES IN MONOCYTIC LEUKEMIA

In monocytic leukemia of the Schilling type there are increased numbers of monocytes (Fig 27) and a paucity of lymphocytes and cells of the myelocytic series. Included among the monocytes are intermediate forms (promonocytes) and monoblasts with nucleoli

and fine reticular chromatin structure (Plate VII D, E; Fig. 28). Atypical and pathologic forms may be present and are difficult to differentiate from cells of the myelocytic series, for these cells have varying types of granules and often have indented or lobular and segmented nuclei. The characteristics to be looked for in the identification of the cells as monocytes are pseudopods (Plate XXVII A, E, H), vacuoles in the cytoplasm (Plate XXVII C, E) and cells with deep nuclear folds and convolutions (Plate XXVII D, E, F, G, H, I). The accuracy of the diagnosis of monocytic leukemia is enhanced by the demonstration of phagocytic activity in some of the cells (Plate XXVII I).

The blunt pseudopods in some of the cells are single and large (Plate XXVII A, E) and in others multiple and small (Plates V E; VI F). Often there is a hyaline ring of ectoplasm which is distinct from the granular endoplasm (Plates V H, I; XXVII E). The nuclei of monocytes in monocytic leukemia are translucent and have a cellophane-like or diaphanous character, enabling the observer to see through the nucleus and to visualize the lobular structure of the nucleus at various depths (Plate XXVII G, H).

Other features of smears from patients with monocytic leukemia are the presence of occasional undifferentiated mesenchymal cells, histiocytes and nucleated red cells. The thrombocytes are decreased Basophils and eosinophils are infrequent or absent.

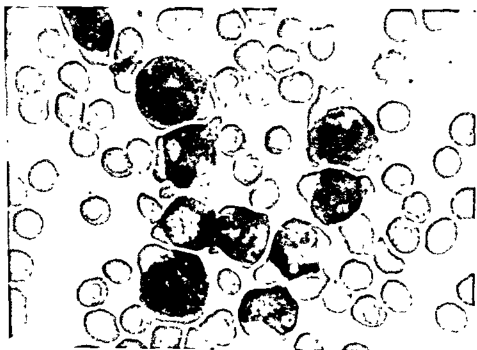


*Figure 28* Photomicrograph. Monocytic leukemia. Blunt pseudopods and indented and folded nuclei The majority of cells are monoblasts and promonocytes

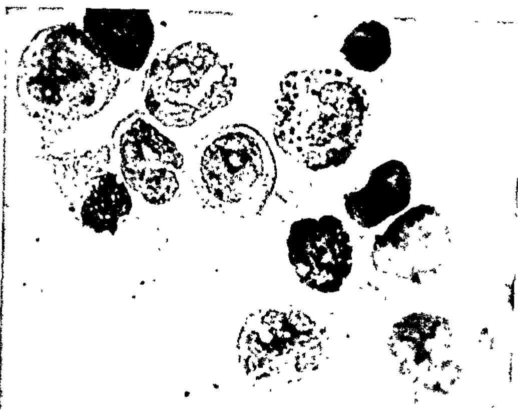


*Figure 29* Photomicrograph Monocytic-myelocytic leukemia (monocytic leukemia—Naegeli type). Indentation and lobulation of nuclei and vacuoles in some of cells simulating monocytes

MONOCYTES IN MIXED TYPES OF MONOCYTIC LEUKEMIA

In malignancies involving the multipotent mesenchymal (reticulum) cells the differentiation may be in multiple directions. One of the most common variants of monocytic leukemia is a condition characterized by the mixture of cells having the morphology of myelocytes as well as monocytes (Fig. 29). This type of leukemia is known as *monocytic-myelocytic leukemia* or the *Naegeli type of monocytic leukemia* in contrast to the more pure form of monocytic leukemia, or *monocytic leukemia* of the *Schilling type*.

There may be differentiation in the direction of histiocytes producing the variant known as *monocytic-histiocytic leukemia*, or a mixture of histiocytes, monocytes and myelocytes *(histiocytic-monocytic-myelocytic leukemia)* The ability to demonstrate in individual cases all combinations of cell types is proof of the close relationship of the different series of blood cells. Often the condition can be recognized as leukemia, but the nomenclature of the type of leukemia is a matter of opinion.

## Plasmocytes

Plasmocytes are subject to marked variations in size, shape, nuclear pattern and color and structure of the cytoplasm. Much of the pleomorphism observed is due to the fact that they are secretory cells which manufacture immune globulins and other proteins. The secretory material may exist in the form of solutions having variable staining reactions, in the form of chromophobic droplets (Plates VII, IX), as granular aggregates, as globules of various size and number and as crystals (Plates XXVIII, XXIX). The delicate reticular structure of the plasmocytes with con-

centric lines parallel to the cytoplasm is usually masked by the intense basophilic stain in the majority of normal plasmocytes, but the reticular structures may be clearly visible and present striking lattice-like and crisscross patterns (Plates VII H; XXVIII).

FLAME CELL

In the majority of plasma cells the basophilic substance (ribosenucleoprotein) predominates over the red staining components of the cytoplasm, but in some instances the concentration of red staining or acidophilic substance is excessive and produces cells with a fiery red color called "flame cells" (Plate XXIX A). The red staining material may be diffusely spread, appear as pools within the cytoplasmic areas (Plate XXIX C) or as a fringe of red at the periphery (Plate XXVIII D).

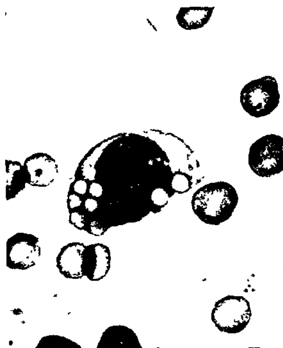


*Figure 30.* Photomicrograph of bone marrow smear of plasma cell myeloma. Russell bodies in plasmocyte.

PLASMOCYTE WITH GLOBULES (RUSSELL BODIES,* EOSINOPHILIC GLOBULES, ACIDOPHILIC GLOBULES)

The secretory material in plasmocytes may be in the form of prominent spherical masses (Fig 30), which vary in size, in number and in staining reaction. The majority of globular masses have an affinity for the red or eosin stain and are therefore called "eosinophilic globules." There is considerable variation in the color of the globules in plasmocytes. Many are practically colorless (Plates XXVIII, XXIX C), others are pink, light blue (Plate XXVIII), purple or green. Globular inclusions may be demonstrable in the nucleus of plasmocytes where they appear as small discrete globules or as grouped spherules contained within the membrane of a larger spherule or as droplets within larger drops (Plate XXVIII). In some cells the globules may be concentrated adjacent to the nucleus (Plate XXIX D), whereas in others the spherical masses may be in the distal cytoplasm (Plate XXIX E).

GRAPE CELL

Cells containing numerous globular inclusions may rupture, liberating groups of spherical masses which may or may not have an attached or adjacent nucleus These grouped globules usually stain delicate pastel shades of pink, blue, lavender or green They often have the appearance of a bunch of grapes and are therefore called "grape cells." These structures are most frequently seen in bone marrow smears of patients with plasma cell myeloma, but may be found in rare instances in various other conditions.

* The eponym "Russell" was applied to the globular inclusions in plasma cells because the reddish globules as seen in the Romanowsky stain resembled the fuchsin-positive or red bodies which Russell described in carcinoma cells (British Medical Journal, 1890, p 1352).

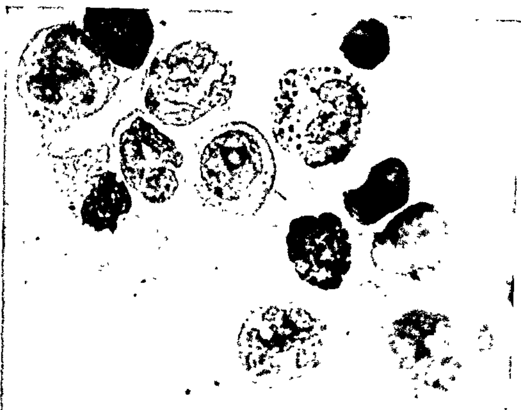


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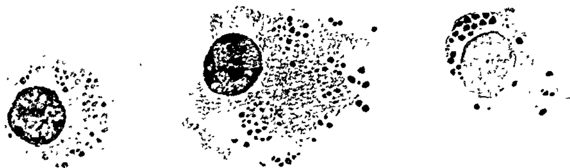


*Figure 31.* Granules in cytoplasm of plasma cells from bone marrow of plasma cell myeloma (Photograph of water color painting by M. Guthrie)

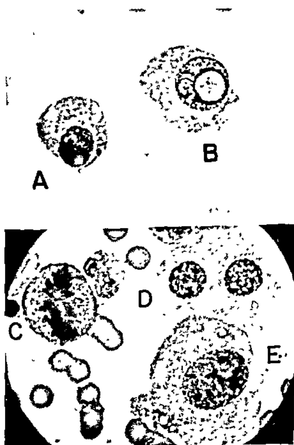



*Figure 32* Photon[illegible] Plasmocytes from
with plasma cell

### PLASMOCYTE WITH GRANULES

Plasmocytes as a class are nongranular, but in smears of bone marrow from patients with multiple myeloma one may encounter a few plasma cells with small globules or aggregated masses of material which have a granular appearance (Plate XXVIII; Fig. 31). These granule-like inclusions are usually acidophilic but may stain blue to purple. It is thought that the granules in plasmocytes are the equivalent of azurophilic granules in lymphocytes and are morphologic manifestations of a secretory activity in which proteinaceous material is manufactured in high concentrations within the cell and is precipitated or condensed in local areas rather than being diffusely distributed.

### PLASMOCYTE WITH CRYSTALS IN CYTOPLASM

The proteinaceous substance within the cytoplasm of plasmocytes may appear in the form of a homogeneous solution or suspension, in the form of globules or lakes or as amorphous granules, and also in the form of crystals The crystals stain intensely and may be bright red or dark blue. They may appear as short pointed rods (Plate XXIX D) or elongated splinters or lancelike structures (Fig 33) These crystalline bodies resemble the structures in immature cells of the granulocytic and monocytic series and, like them, are called "Auer bodies" or "Auer rods "

### PLASMOCYTE WITH BIZARRE SHAPE

In multiple myeloma the plasmocytes may have extremely long, tenuous and curved extensions of cytoplasm which are often fragmented and fill the background with cellular debris (Plate XXIX; Figs 34, 35) The peculiar shape of the plasma cells is in part due to the tearing away of the fixed tissue cells at the time of aspiration, and in part due to the discharge of globular material or pools of secretory substance when the cytoplasmic membrane is ruptured. The reticular fibrils which enclosed the secretory material are left behind in much the same manner as the pulp of an orange is left after the juice has been *mechanically expressed.*

### PLASMOCYTE WITH MULTIPLE NUCLEI

Double nuclei in plasmocytes are common in smears of bone marrow of normal individuals. In malignancies of the plasmocytic series there may be giant forms with various types of polyploid nuclei, multipolar nuclei (Plate XXIX B; Fig. 32) and cells with odd numbers of nuclei which may have similar or different chromatin patterns (Fig 35, left).

### PLASMOCYTE WITH PROMINENT NUCLEOLI (THE "MYELOMA CELL")

In multiple myeloma one may find many plasmoblasts which have abnormally large and prominent nucleoli (Fig. 32). Such cells have been called "myeloma cells " Because the anaplastic and undifferentiated plasma cells are morphologically different from mature and normal plasmocytes, many authors have assumed that these cells are not plasma cells and should be given a separate name. This concept in relation to the plasma cells is no more justified than it would be to assume that the anaplastic cells of a carcinoma are not epithelial cells because the cells are not keratinized or arranged in a syncytial or glandular pattern The term "myeloma cell" should be dropped, for it adds nothing and creates confusion

### PLASMOCYTE SIMULATING LYMPHOCYTE (TURK'S IRRITATION CELL)

In the older hematologic literature, nongranular cells with a dark blue cytoplasm and an eccentric nucleus which were morphologically borderline between lymphocytes and

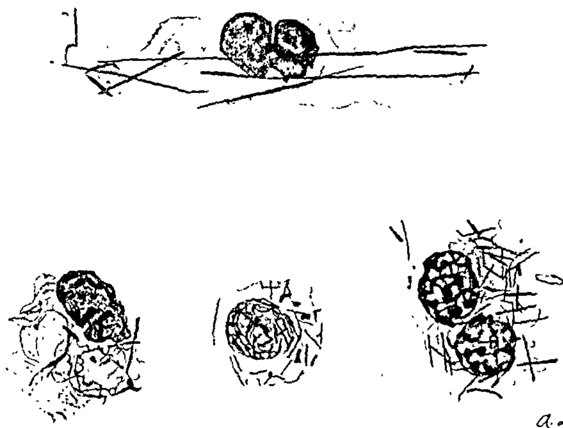



*Figure 33* India ink drawing Auer rods in plasmocytes in bone marrow smear from a patient with plasma cell myeloma (Courtesy of Dr Ralph L. Engle, Jr., New York City, N Y.)

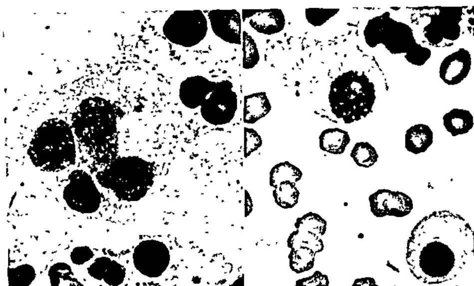


*Figure 34* Photomicrograph of smears of bone marrow of plasma cell myeloma. *Left,* Multinucleated plasma cell! Cytoplasmic streamers Increased protein in background with cellular debris. *Right,* Two plasmocytes, one with irregular cytoplasmic margins Rouleaux formation.



*Figure 35* Photomicrograph of bone marrow, plasma cell myeloma *Left,* Polyploid nucleus with nuclei of different sizes *Middle,* Three plasmocytes, one with double nucleus and one with herniation of nulcear chromatin through nuclear membrane. *Right,* Plasmocyte with hairlike cytoplasmic extensions

the typical "Marschalko plasma cells" were called "Türk's cells" or "Turk's irritation cells." Such cells are found in small numbers in the peripheral blood in chronic infections and in conditions in which there is active *antibody formation.* It is recommended that the term "Türk's irritation cell" be eliminated and that the cell be tallied as a "plasmocyte" or as an "atypical lymphocyte."

## METASTATIC MALIGNANT CELLS VERSUS PLASMA CELLS

Cells of reticulum sarcoma (malignant reticuloendotheliosis) and metastatic carcinoma cells may closely resemble plasma cells, for such cells may have *an eccentric nucleus,* blue cytoplasm and irregular shapes. Prostatic, breast and other tumors metastatic to bone may have the clinical features of multiple myeloma such as chronic and progressive course, severe anemia, bone pains, pathologic fractures and radiologic evidence of osteolytic lesions. The smears of bone marrow reveal cells which are interpreted as malignant, but it may be difficult to tell whether the malignant cells are plasmocytes or some other cell type. The difficulty is compounded by the fact that plasmocytes are often increased as reactive cells at the site of metastatic malignancy. The examination of sections of fixed tissue and the appraisal of the entire picture may help, but occasionally there is a difference of opinion even after *autopsy*

The differential diagnosis between plasmocytes, sarcoma cells and metastatic malignant cells is best made by a careful study of thin smears Malignant cells other than plasma cells are *not likely to have prominent light* areas next to the nucleus and do not have a lamellar cytoplasmic reticular *pattern.* Secretory bodies in the form of Russell bodies, lakes of red-staining material at the periphery and crisscrossing fibrillar structures are plasmocyte characteristics which are seldom observed in malignancy of a nonplasmocyte type.

## PLASMOCYTES IN PLASMA CELL MYELOMA (MULTIPLE MYELOMA)

Malignancy involving the plasma cells may be *in* the form of a localized and relatively benign tumor (plasmocytoma), a systemic disease with generalized involvement of the marrow cavity with extension to other tissues and with few plasmocytes in the peripheral *blood (plasma cell myeloma),* or a systemic disease with a large number of plasmocytes in the peripheral blood (plasma cell leukemia). There is no sharp differentiation between these various forms. The same patient may *show at successive stages in the process all* three variants.

In the early stages of multiple myeloma the islands of proliferative plasmocytes are unevenly distributed and aspiration of marrow at one site will reveal no significant increase in plasmocytes, whereas in another area plasmocytes may be the predominant cell. As a rule, by the time the patient has symptoms and signs due to myeloma, the plasmocytes will be increased in all portions of the bone marrow. In the majority of cases of plasma cell myeloma the percentage of plasmocytes *in the bone marrow smears is greater than 10* per cent In conditions other than plasmocytoma, it is rare to have more than 10 plasma cells per 100 nucleated cells The diagnosis, *however, is not* based upon numbers alone but on the morphology of the individual cell.

Features which favor malignancy of the plasmocytic cells are variability in size and *shape, mitotic* figures, *multilobular mitosis,* giant cells, and increased number of immature forms with prominent nucleoli (the *myeloma cell*), aberrant nuclear material in cytoplasm and retention of secretory products Associated morphologic phenomena are striking rouleaux of red cells, bluish background, and basophilic cellular debris (Figs 32, 34, 35).

## Miscellaneous

### CLOVER LEAF NUCLEI

Nuclei of cells in *specimens of oxalated blood* frequently assume bizarre and clover-leaf shapes. This abnormality in the shape of the nucleus affects lymphocytes and monocytes as well as cells of the myelocytic series (Fig. 36) and often makes it difficult to perform an accurate differential count Because of the importance of nuclear shape in the differential diagnosis of the various cell types, it is obvious that one should never use oxalated blood in the diagnosis or differential diagnosis of diseases of the hematopoietic organs.



*Figure 36.* Peripheral blood smear of oxalated venous blood Hyperlobulation and distortion of nuclear pattern Left upper and left lower cells are neutrophils, right upper a monocyte and right lower a lymphocyte

### CELL WITH NUCLEAR CLEFTS AND CLEAVAGE LINES

During the process of mitosis and cell division, the cleavage of the nucleus may be partial or incomplete Instead of two or more distinct nuclei, there is a partial cleavage or splitting of the nucleus The cleavage lines are usually straight and resemble "cracks" in the nucleus (Fig 26). In some cells the cleavage line may traverse the entire width of the nucleus In others there is a short straight line In multipolar mitoses there may be multiple dividing lines

Cells with nuclear clefts are most frequently seen in smears from patients with lymphocytic leukemia (Plate XXVI G, H) and giant follicular lymphoblastoma, but clefts of the same type may be seen in leukemic cells of the myelocytic and monocytic types. The finding of such cells is presumptive evidence of a leukemic process, but is not diagnostic of malignancy Nuclear cleavage lines may be found in smears of normal infants, following treatment with x-ray or radioactive substances, and in rare instances in numerous other conditions

### UNDIFFERENTIATED CELL WITH INDENTED NUCLEI (RIEDER CELL)

In malignancies of the hematopoietic organs one often sees evidences of asynchronism between the shape of the nucleus, the chromatin structure of the nucleus and the color and granularity of the cytoplasm One of the common variants is the indentation and lobulation of the nucleus in cells which otherwise would be identified as "blasts" or undifferentiated cells. Such atypical cells have been designated as "Rieder cells." The abnormal folds and lobules in undifferentiated cells are not specific and may be found in lymphocytic leukemia as well as in monocytic and myelocytic leukemia

*Chapter VI*

# THE LUPUS ERYTHEMATOSUS CELL

THE "L E." or "lupus erythematosus" cell is a phagocytic leukocyte, usually a segmented neutrophil which contains within its digestive vacuole a mass of lysed, homogeneous nuclear material which is Feulgen-positive and which has a reddish or purple color when stained by the Wright's method (Hargraves, Richmond and Morton Proc. Staff. Meet, Mayo Clinic, *23*:25, 1948, Hasserick and Sunberg: J Invest. Dermat. *11*:209, 1948). Cells having this morphology (Plate XXII I) are most often seen in patients with systemic lupus erythematosus

The so-called "L.E." cell may be demonstrable in fluids from serous cavities and blister fluids of patients with systemic lupus erythematosus when the smears are made immediately after aspiration, but they are seldom demonstrable in blood and bone marrow smears made at the time of collection The lysis of some of the leukocytes and phagocytosis of the lysed nuclear material by other leukocytes requires time to develop outside of the body or outside of the active circulation. The formation of L.E cells is due to or associated with a peculiar type of gamma globulin in the plasma of affected individuals It can be demonstrated by the

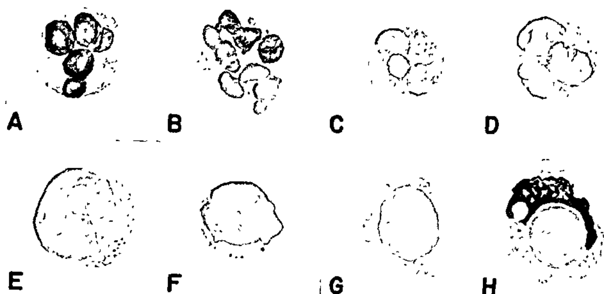



*Figure 37.* A sequence in the development of the L E. cell *A*, Segmented neutrophil with beginning lysis of nucleus *B*, More advanced nuclear lysis. *C, D*, Advanced nuclear lysis with swelling of nuclear lobes and light stain (without pyknosis) *E, F*, Neutrophil undergoing lytic changes *G*, Pre-L.E. cell with lytic nucleus and disintegrating cytoplasm *H*, L E cell showing phagocytosis of lysed spherical nucleus by neutrophil

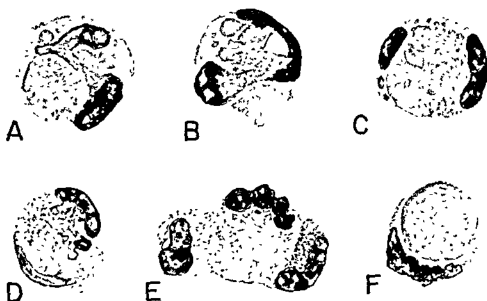



*Figure 38.* Stages in development of L E cells. *A, B, C*, Stages in lysis of nuclear chromatin in neutrophils *D*, End stage in lysis of nuclear chromatin *E*, Grouping of three granulocytes around lysed nuclear structure *F*, Phagocytosis of partially lysed nuclear chromatin by another neutrophil producing the L.E cell

addition of plasma of patients with lupus erythematosus to cells of normal individuals or to the cells of other species.

The initial or "pre-L.E. stage," which begins a few minutes after the blood is drawn, is characterized by an accentuation of the chromatin markings in one or more lobes of a given neutrophil, followed by a swelling and merging of the chromatin strands (Fig. 37 A-D). The individual lobes become larger, more homogeneous, and take a less intense color. Ultimately the various lobes may be fused into a globular mass (Fig. 37 E, F, G). The nuclei of some of the cells undergo granular or globular disintegration (Fig. 38 A-E). The chromatolytic process appears first to involve the more centrally located portions of the nucleus (Fig. 38 A-D), but later the entire nucleus is lysed Associated with the lytic changes taking place in the nucleus, the granular cytoplasm likewise undergoes dissolution and the cytoplasmic margin becomes indistinct (Figs 37, 38).

In the second or "rosette stage" of the L.E. process, viable and mobile leukocytes which have not undergone lytic processes, or have not been injured to the same degree, are attracted to the cells which have undergone advanced nuclear and cytoplasmic degenerative changes. They cluster around the aggregates of nuclear material and extend their pseudopods along the margins of the nuclear masses (Figs. 38, 39, 40, 42).

The third stage in the process is the ingestion of the mass of lysed nuclear material by the phagocyte producing the L E cell (Figs. 37, 38, 39, 40, 41). Sometimes one neutrophil will ingest one portion of the nuclear material and another phagocyte the other portion, or two neutrophils will attempt to phagocytize the same mass (Fig. 41). Often a given cell will phagocytize multiple portions (Fig 41 C, D). After the nuclear material is taken into the digestive vacuole of the cell, the phagocytized mass tends to become more compressed, darker and more homogeneous. The L.E. inclusion bodies vary considerably in their staining intensity. Some are quite light and pink while others are dark purple. The inclusions vary in size but are usually larger than erythrocytes and as a rule are 10 to 30 micra in diameter. The ingested material is usually in the form of a single sphere, but it may be composed of two or more compressed globules (Figs 39, 41). The margins of the L.E. inclusion are smooth. On cursory inspection the L.E. body appears to be quite homogeneous and has a velvety appearance, but on careful focusing under oil immersion it is seen that there are lighter

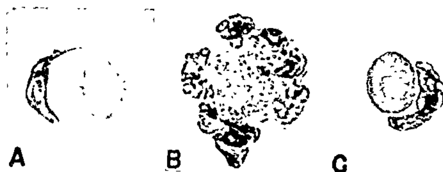



*Figure 39* *A*, L E. cell *B*, L E rosette *C*, L.E cell.

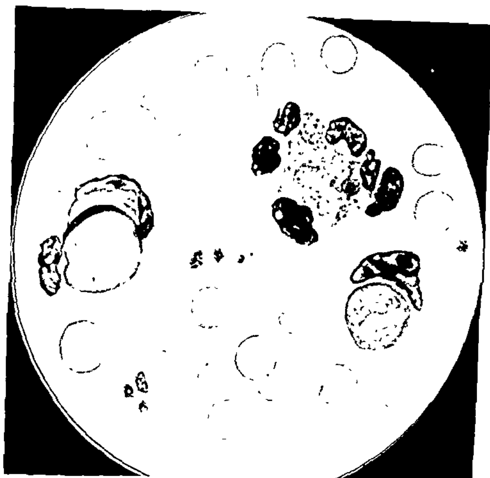


*Figure 40.* L.E. cells and L.E. rosette (Water color painting by Doris McGowan)

areas interspersed between solid and more darkly stained portions (Figs 39 C, 40) Many of the inclusions have ill-defined patterns but do not have distinct chromatin markings or areas of lumpy basophilia. In occasional L.E. inclusions there may be a slight increase in the density of the staining at the periphery of the mass (Fig. 40), but as a rule the stain is uniform throughout or darker in the center, and there is no significant condensation of stain at the margin.

The phagocytic L.E. cell with its ingested material is usually larger than are adjacent nonphagocytic neutrophils. The nucleus is pushed to one side and appears as a flattened wreath around the mass. The nucleus of the phagocytic cell often undergoes "pre-L E" changes of the same sort as occurred in the original cells undergoing lysis (Fig 42) The L.E. inclusion, and the cell containing the inclusion, ultimately disintegrate as the blood is allowed to stand (Fig 42).

The nuclei which are most frequently involved in the lytic process are the nuclei of neutrophils, but the nuclei of lymphocytes, myelocytes, plasmocytes and other cells are likewise subject to chromatolysis. Segmented neutrophils are the cell types which most frequently serve as phagocytes, but monocytes, eosinophils, band neutrophils and metamyelocytes may participate in the phagocytic process

The reported incidence of demonstrable L.E. cells in patients with proved lupus erythematosus varies in different series depending on the technique used, the diligence with which cells are looked for, the skill of the examiner and the number of examinations

made. The cells tend to become more frequent and typical in the later stages of the disease than at the beginning of the illness. A few patients who have classical symptoms and signs and anatomic confirmation of the disease at autopsy may fail to reveal L.E. cells in smears of blood and bone marrow on frequently repeated examinations. A negative test, therefore, does not exclude the disease.

The number of L.E. cells in different preparations is unpredictable. In the more typical cases there may be more than one L E. cell per 100 neutrophils, and often multiple cells in a given oil immersion field. In other patients long search has to be made in order to find the characteristic forms.

The L.E. cell is not specific for lupus erythematosus although the correlation between cells called L.E. cells and lupus erythematosus is high. L.E. cells have been reported in penicillin and Apresoline reactions, serum sickness, acquired hemolytic anemia, miliary tuberculosis, fungus infections, scleroderma, rheumatoid diseases, dermatitis herpetiformis, multiple myeloma and amyloid disease L E. bodies can be produced experimentally (Fig. 43) by mixing human blood cells with the serum of rabbits immunized by injections of human leukocytes (Finch et al.: J. Lab Clin. Med. *42*:567, 1953; Zimmerman et al.: Blood *8*: 651, 1953).

Phagocytosis of nuclei and nuclear fragments of other cells is a common phenomenon in all specimens of heparinized bone marrow and in specimens of defibrinated blood and blood to which anticoagulants have been added Nucleophagocytosis occurs in the blood of normal individuals and in numerous

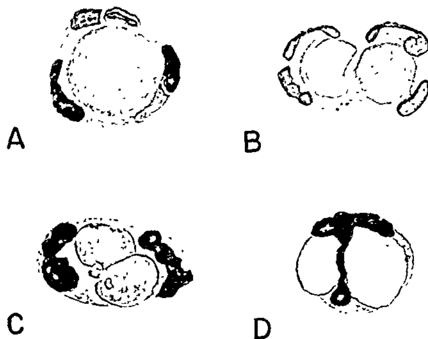



*Figure 41* Variants of L E cell *A*. Spherical L E body with fairly homogeneous structure. Beginning lysis of one of lobes of phagocytizing neutrophil *B*. Two adjacent L E bodies phagocytized by neutrophils *C*. Bilobed L E body phagocytized by two neutrophils. *D*. Two L E. bodies in neutrophil

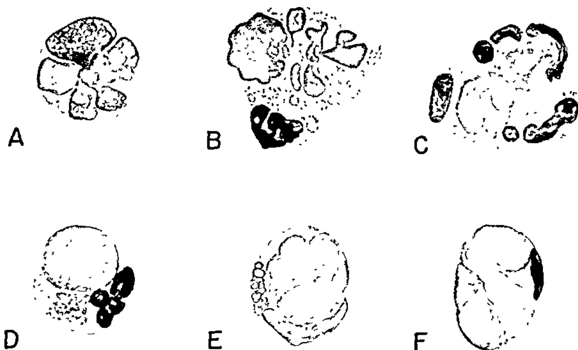



*Figure 42.* Variants of L E phenomena *A*, Pre-L E. cel'; lysis of nucleus with clover-leaf swelling *B*, Partially lysed nucleus of segmented neutrophil with intact neutrophil adjacent *C*, Lysed nuclear chromatin surrounded by neutrophils. *D*, Neutrophil containing L E body with pyknotic nucleus, beginning nucleolysis. *E*, Degenerated L.E cell with lysed nucleus and vacuoles containing multilobulated L E. body *F*, Degenerated L E cell with partially lysed nucleus containing multiple L.E bodies

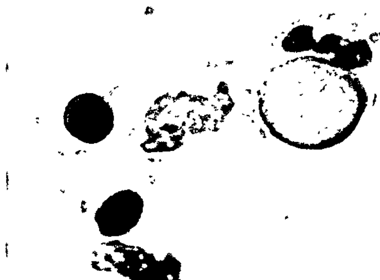


*Figure 43* Neutrophil with "L E -like" body experimentally produced (Courtesy of Dr. S C Finch, and J Lab & Clin Med, 1953.)

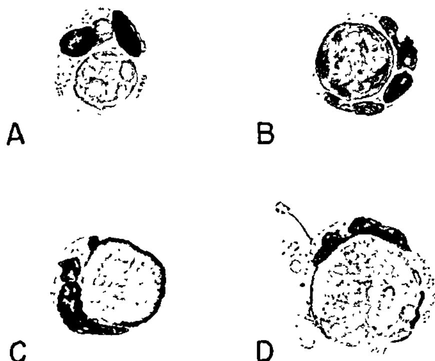



*Figure 44* "L E -like" cells (from patients without lupus erythematosus) *A*, Segmented neutrophil with phagocytized partially lysed nucleus with residual pyknosis *B*, Segmented neutrophil with phagocytized partially lysed nucleus with linear nuclear chromatin structure *C*, Neutrophil with phagocytized spherical body having linear chromatin pattern and prominent marginal zone of basophilia *D*, Neutrophil containing large spherical body resembling L.E inclusion but having residual nuclear structure.

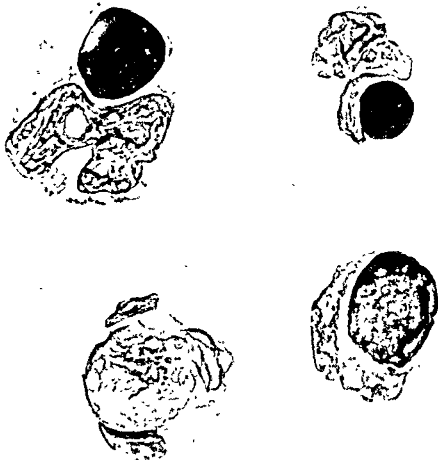


*Figure 45.* Phagocytized nuclei. "Tart cells" (confused with L E cells) (Photograph of water color painting by Doris McGowan )

conditions other than lupus erythematosus. The phagocytic cell in "non-lupus erythematosus" conditions is usually a monocyte. The nucleus which is phagocytized is usually not completely lysed but maintains evidence of nuclear structure such as linear chromatin pattern, uneven basophilia, increased intensity of stain at the margin or diffuse basic stain (pyknosis) (Figs. 44, 45; Table 8). Such cells, called "tart cells" by Hargraves and associates, are readily differentiated from true L.E. cells when the phagocytizing cell can be identified as a monocyte and the nuclear markings of the inclusion are distinct (Plate XXII H). The difficulty lies in the identification of individual cells when the degree of nucleolysis is borderline or questionable. In the so-called "tart cell" there are all degrees of nucleolysis, and in the so-called L.E. cells the nuclear mass is not always completely homogeneous and devoid of nuclear structure. It is impossible in all cases to differentiate L.E. cells with complete lysis of nucleus from "non-L.E." or "tart cells" with almost complete lysis (Figs. 39, 40, 43, 44, 45)

In addition to nonlysed or incompletely lysed nuclei in the cytoplasm of phagocytes (tart cells),* there are numerous other types of spherical objects which simulate L E. inclusions and which must be differentiated The "degenerative vacuoles" or "digestive vacuoles" within the cytoplasm of neutrophils are chromophobic or stain very lightly in contrast to L E bodies which stain dark red or purple. Phagocytized erythrocytes may be prominent in some preparations Their red color, homogeneous appearance and round shape superficially resemble L.E. bodies. Phagocytized red cells have the color of nonphagocytized erythrocytes in the same field and lack the purple and bluish tints of nuclear material.

* The word "tart" was not used because the cells resembled cookies with a fruit or jelly inside, because the cells had a sour or acrid taste, or because they were female cells of ill repute, but because they resembled the cells found in the blood smears of Mr Tart who was a patient at the Mayo Clinic

**Table 8. Differential Diagnosis of Phagocytized L.E. and "Non-L.E." ("L.E.-like") Inclusions**

| | PHAGOCYTIZED "NON-L.E." INCLUSIONS | PHAGOCYTIZED L.E. INCLUSIONS |
|---|---|---|
| Phagocytic cell usually involved | Monocyte | Neutrophil |
| Linear chromatin structure | Demonstrable | Indistinct or absent |
| Pyknosis | Present | Absent |
| Peripheral condensation | Frequent | Rare |

Secretory globules in plasmocytes (Russell bodies) or in metastatic malignant cells may be indistinguishable from L E. cells. Such cells, however, are rare. The diagnosis is usually obvious on the basis of other cells in the same preparation Other rare morphologic variants which may simulate L.E. cells are amyloid inclusions and immature cells with degenerated nuclei surrounded by a dark blue crescent of cytoplasm.

In the majority of instances it is possible for the examiner who is requested to look for L E cells to give one of the following reports:

1. No L E. cells seen
2. Nucleolysis and nucleophagocytosis of the type seen in lupus erythematosus demonstrable in — per cent of neutrophils.
3 No typical L.E cells seen Occasional "L.E.-like" cells demonstrable in — per cent of neutrophils.

*Chapter VII*

# PATHOLOGIC MEGAKARYOCYTES AND THROMBOCYTES

In THROMBOCYTOPENIC states the examination of the bone marrow is often of value in diagnosis and in deciding whether or not splenectomy is indicated. Splenectomy is contraindicated in those conditions in which the megakaryocytes are significantly decreased (amegakaryocytic thrombocytopenia) and in thrombocytopenic conditions in which there are numerous megakaryocytes which are producing granular platelets

The estimation of the number of megakarocytes is unreliable in thin marrow smears in which there is considerable dilution with peripheral blood Their number is most accurately evaluated in tissue sections. The examination of the marrow smears in thrombocytopenic states should include a differential megakaryocyte count of at least 25 cells. The giant cells of the bone marrow should be catalogued as megakaryoblasts, promegakaryocytes, megakaryocytes without granular platelets (intermediate forms), megakaryocytes with platelet formation (metamegakaryocytes) and naked nuclei (Plates X, XI).

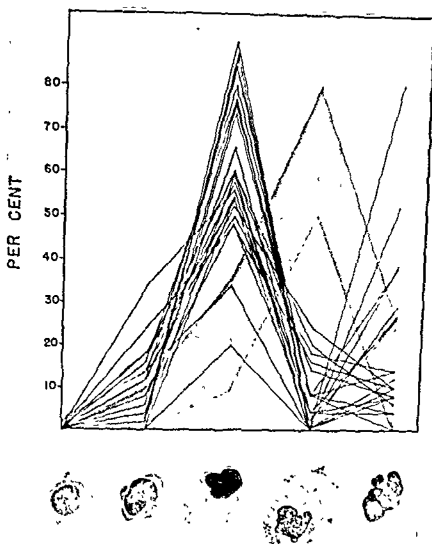



*Figure 46* Frequency distribution of megakaryoblast, promegakaryocyte, megakaryocyte without platelet production, megakaryocyte with platelet production and naked nuclei in sternal bone marrow smears of 22 patients with idiopathic thrombocytopenic purpura in the acute stage The variation in the percentage of different maturation stages of megakaryocytes in normal individuals is represented by the shaded area (Diggs and Hewlett *Blood 3 1090, 1948*)

In idiopathic thrombocytopenic purpura the number of megakaryocytes is normal or increased. The majority of the megakaryocytes are in the intermediate stage and do not have demonstrable marginal thrombocytes (Fig. 46). The number of naked nuclei is increased. It is thought that the factors which cause a lysis or destruction of platelets in the peripheral blood also cause a dissolution of the cytoplasm of megakaryocytes in the bone marrow and in the capillaries of the lung.

Megakaryocytes are increased in the bone marrow in chronic myelocytic leukemia, polycythemia and other myeloproliferative diseases, and may appear in the peripheral blood in these conditions.

In megakaryocytic leukemia there is a significant increase in megakaryoblasts, promegakaryocytes and pathologic forms in the bone marrow. Megakaryocytes and atypical cells having the characteristics of megakaryocytes may also be demonstrable in the blood smears, with or without an increase in thrombocytes. Nucleated red cells and immature cells of the myelocytic series are usually present.

Giant cells of the bone marrow are decreased in monocytic, lymphocytic and stem cell leukemia, in plasma cell myeloma and in the terminal stages of myelocytic leukemia. Megakaryocytes are also sparse in hypoplastic and aplastic anemias

The size, shape and structure of thrombocytes in the peripheral blood are subject to wide variations in different pathologic conditions. There may be occasional thrombocytes or cytoplasmic fragments of megakaryocytes which are larger than erythrocytes, and cylindrical forms which may be 50 or more micra long. Thrombocytes may be granular or hyaline or may have a granular center and a nongranular margin.

Numerous studies have been made by various workers in order to establish the specificity and significance of the morphologic variants of the thrombocytes. The few platelets that are present in the circulating blood of patients with thrombocytopenic purpura are likely to be larger than normal, and the thrombocytes of some patients with thrombasthenia are said to be pleomorphic and more intensely stained than normal (Stefanini and Dameshek: *The Hemorrhagic Disorders,* Grune and Stratton, 1955), but these changes are not constant or specific.

*Chapter VIII*

# PATHOLOGIC CELLS: MISCELLANEOUS

## Pathologic Fixed Tissue Cells

The cells of the blood and bone marrow, under physiologic conditions and in normal individuals, are thought of and are classified in one of two categories, as "fixed tissue cells" or as "free blood cells." The degree of fixation, however, is not the same for all cells (Table 9) Some cells such as fibroblasts with collagen fibers, reticulum cells with reticulum fibers and osteocytes and chondrocytes are so firmly anchored by interlacing fibers or intercellular cement substances that they cannot spontaneously escape. These cells are aspirated with great difficulty Other cells such as granular histiocytes and plasmocytes do not appear in the peripheral blood smears of normal individuals, but are frequently present in the circulating blood in pathologic conditions These semifixed cells are numerous in smears of aspirated bone marrow. In bone marrow smears and in diseased states it is therefore necessary to develop a concept of intermediate cells which are partially fixed and partially free (Table 9).

**Table 9. Bone and Bone Marrow Cells Arranged According to Degree of Fixation**

| | CELL TYPE | REMARKS |
|---|---|---|
| *Fixed Cells:* | | |
| | Chondrocyte | Readily demonstrable in tissue sections |
| | Fibroblast | |
| | Endothelial cell | Seldom demonstrable in smears of material aspirated by needle from the marrow |
| | Reticulum cell | |
| | Osteocyte, osteoblast, osteoclast | |
| | Fat cell | Not present in peripheral blood smears |
| | Tissue basophil (mast cell) | |
| | Tissue eosinophil | |
| *Semifixed Cells* | | |
| | Undifferentiated mesenchymal cell (hemohistioblast) | Present in smears of normal bone marrow |
| | *Granular histiocyte* | |
| | Phagocytic fixed tissue histiocyte | Not present in smears of peripheral blood from normal individuals |
| | Plasmocyte | |
| | Megakaryocyte | |
| | Immature leukocytes | Demonstrable in peripheral blood smears in *proliferative and malignant conditions* |
| | Nucleated red cells (erythroblasts) | |
| *Free Cells:* | | |
| | Phagocytic ameboid histiocyte | |
| | *Mature leukocytes* | Present in peripheral blood smears of normal individuals |
| | Erythrocytes | |
| | Thrombocytes | |

The following descriptions deal with cells of the semifixed types which are loosely attached and are demonstrable in marrow smears and occasionally in the circulating blood. The immature cells of the leukocytic, erythrocytic and thrombocytic series were discussed in the preceding chapters.

## GRANULAR HISTIOCYTE (FERRATA CELL, TISSUE NEUTROPHIL) IN VARIOUS DISEASES

One of the most ubiquitous of the semifixed tissue cells of the bone marrow (Table 9) is the granular histiocyte. This large and irregularly shaped cell with ample cytoplasm, reddish bacilliform granules and granules in beadlike chains, and round or oval nucleus with coarse chromatin pattern (Plates XII, XIII) constitutes approximately 1 per cent of the nucleated cells in smears of bone marrow from normal individuals This cell is closely related to the reticulum cells (reticulum cells with reticulum fibrils which have an affinity for the silver stain) on one hand and the neutrophilic myelocytic and monocytic cells on the other. In pathologic conditions in which the cells may be atypical it may be impossible, on morphologic grounds alone, to identify all cells as granular histiocytes, progranulocytes, neutrophilic myelocytes or monocytes, for there are transitional forms which have the features of all three cell types.

The granular histiocytes are increased in myeloproliferative diseases, including chronic myelocytic leukemia, myelosis (agnogenic myeloid metaplasia), and polycythemia vera. In chronic myelocytic leukemia these large cells are readily demonstrable in the smears of peripheral blood as well as in the bone marrow Granular histiocytes are increased in the bone marrow in infections. In chronic infectious states there is often an accompanying increase in monocytes, plasmocytes, eosinophils and lymphocytes. In diseases characterized by granuloma formation such as tuberculosis, undulant fever, sarcoid and Hodgkin's disease, the histiocytes of the granular type are often conspicuous They are likewise increased in conditions in which there is neutropenia with an arrest in the maturation of neutrophilic leukocytes as in agranulocytosis, virus diseases, chemical poisoning and overexposure to radioactive substances It is obvious that a cell which is increased in so many different conditions has little differential diagnostic value.

The cells of the granular histiocytic type may be involved in a malignant process, producing the disease known as *histiocytic leukemia* * In this rare type of leukemia, the predominant pathologic cells in the bone marrow and peripheral blood smears maintain the characteristics of granular histiocytes. They are differentiated from typical monocytes by the fact that they have cytoplasmic rods and granules in chain formation, spindle and pointed shapes of fixed tissue cells rather than the blunt pseudopods of actively motile cells Transitional forms between histiocytic and monocytic leukemia of the Schilling type occur in which it is a matter of opinion among hematologists which name to apply to the disease. The authors prefer to recognize similarities rather than to be dogmatic about differences. In borderline situations of this type it is recommended that the terms "histiocytic-monocytic" or "monocytic leukemia of the histiocytic type" be used.

* Malignancies of the less differentiated and more fixed histiocytes or reticulum cells are designated as "reticulum cell sarcomas" Aspiration of the bone marrow or tumor mass in this disease is of limited value for the definitive cells are so firmly attached that they are obtained in small numbers in marrow smears True reticulum cells with reticulin fibrils seldom are demonstrable in the peripheral blood Nonlipid reticuloendotheliosis or Letterer-Siwe's disease is considered by many as a generalized proliferative disease of childhood involving the reticulum cells of the hematopoietic tissues and closely related to systemic reticulum cell sarcoma

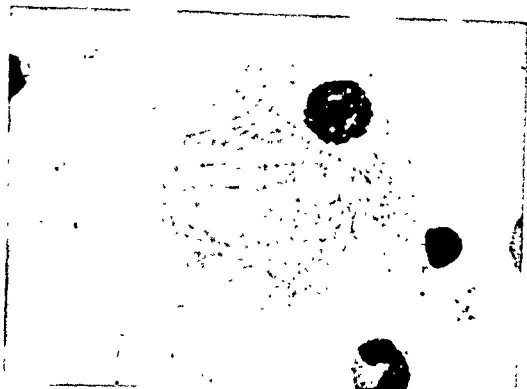


*Figure 47* Photomicrograph Gaucher cell in bone marrow smear.

## GAUCHER CELL (LIPID HISTIOCYTE OF THE KERASIN TYPE)

Gaucher cells are fixed tissue histiocytes which contain within their cytoplasm multiple round and linear chromophobic inclusions The cells vary considerably in size and shape but are usually large (25 to 50 micra in diameter) There is abundant light staining cytoplasm which has a crinkled or shredded appearance The nuclei are small and round with nonspecific chromatin structure There may be giant forms with multiple nuclei The cells have a tendency to occur in groups Gaucher cells are best seen in tissue sections and in the thicker portions of bone marrow smears, especially at the ends and margins. They may be more readily demonstrable in smears of marrow concentrate (Fig 48) than in smears of marrow without concentration

The cytoplasmic pattern is quite variable in different cells. The most distinctive cells are filled with long rodlike or slightly bent structures with rounded or pointed ends (Plate XXX; Fig. 47). The linear cytoplasmic structures may overlie the nucleus and appear as light lines across the nucleus. In other cells the cytoplasmic inclusions are less elongated and more spherical, giving to the cell a foamy appearance (Fig. 48) The cytoplasm has a delicate reticular structure which takes a light blue stain.

## NIEMANN-PICK CELL (LIPID HISTIOCYTE OF THE PHOSPHATIDE TYPE)

In Niemann-Pick's disease smears of material aspirated from the bone marrow and the spleen reveal fat-laden histiocytes which give a positive reaction with Sudan stains. *These cells are scattered throughout the smear* in small numbers or may appear in groups. As a class, they are large cells with abundant cytoplasm and with a relatively small, round

or oval nucleus (Plate XXX). The nucleus is usually single, but double nuclei are not uncommon. The cells resemble the ordinary fat cell, but differ from it in that the globules of fatty material are small and relatively uniform in size. The chromophobic droplets of fatty material are enclosed in the interstices of the reticular net, giving to the cell a foamy appearance Niemann-Pick cells do not have the linear type of inclusions and the wrinkled appearance of Gaucher cells.

Some of the Niemann-Pick cells have globules which are more numerous at the periphery of the cytoplasm than in the center of the cell The juxtanuclear area in such cells is relatively homogeneous and more acidophilic than the marginal zone

Vacuolated monocytes, lymphocytes and neutrophils in the peripheral blood may be demonstrable in some cases of Niemann-Pick's disease. These cells are not specific or diagnostic but have some confirmatory value.

## HISTIOCYTES IN OTHER TYPES OF PROLIFERATIVE DISEASES OF THE HISTIOCYTIC SYSTEM OFTEN ASSOCIATED WITH LIPID STORAGE

In addition to entities such as Gaucher's disease and Niemann-Pick's disease in which characteristic cell types may be demonstrable, there are numerous other conditions characterized by local or systemic proliferation of histiocytic cells with variable numbers of lipid-laden cells of a nonspecific type. These include nonlipid histiocytosis (Letterer-Siwe's disease) (Fig 49), infantile amaurotic familial idiocy (Tay-Sachs disease), eosinophilic granuloma of bone, histiocytosis of the cholesterol type (Hand-Schuller-Christian disease) and xanthomatosis. In these conditions diagnostic cells are not demonstrable in smears of aspirated material from bone marrow, lymph nodes or spleen. The diagnosis is based upon the combined clinical picture,

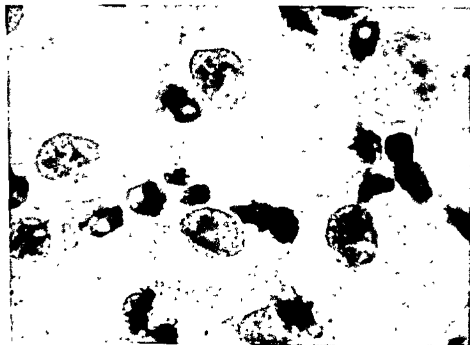


*Figure 48.* Gaucher's cells (Wright's stain). Photomicrograph of marrow concentrate

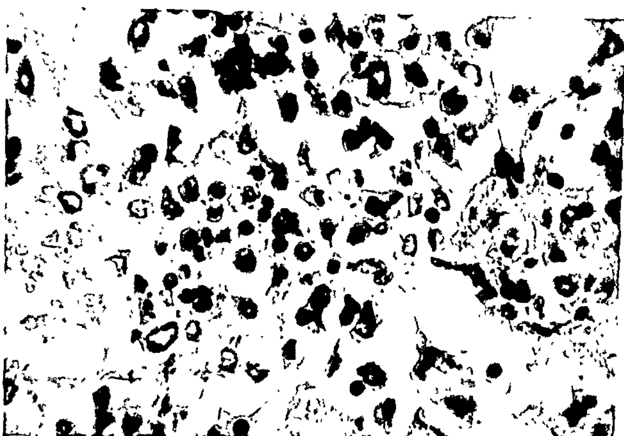


*Figure 49.* Bone marrow section of nonlipid histiocytosis (Letterer-Siwe's disease) The normal bone marrow cells are replaced by fixed tissue cells.

radiologic findings, biopsies, clinical course and response to therapy, together with confirmatory hematologic findings

As additional cases of the so-called "lipoid storage" or "lipid storage" diseases are studied, it becomes apparent that the new cases do not follow the clear-cut patterns described by the authors who observed the first cases. Combinations and transitions are encountered It is not known whether diseases within this group are fundamentally due to abnormalities in fat metabolism or to a proliferative reaction of histiocytic cells to various etiologic agents Whether or not the lipid substances are produced elsewhere in the body and phagocytized by the fixed tissue cells or are formed *in situ* within the cells, it is obvious that the material within the cells which gives them a fat-laden or "foamy" appearance is in excess of need and that the cell is unable to utilize the accumulated material or to dispose of the stored inclusions as quickly as they are formed.

Fixed tissue cells or ameboid cells containing variable amounts of globular material which give the staining reaction of fat are found in the bone marrow and in smears of aspirated splenic material in numerous conditions other than the so-called "lipid storage diseases" The spherical inclusions in the "lipophages" or "fat-laden macrophages" may be colorless or have pastel shades of color from faint pink to blue and green in Wright's stained smears.

## REED-STERNBERG CELL OF HODGKIN'S DISEASE (LYMPHOGRANULOMATOSIS)

The cells described as Reed-Sternberg cells and considered as one of the characteristic reactive cells in this type of lymphoma are giant polyploid mesenchymal (reticulum) cells. The more typical cells have a large hyperlobulated nucleus and ample cytoplasm.

The nucleus may be round, indented or segmented. The chromatin markings are distinct, with a sharp separation between chromatin and parachromatin Multiple large nucleoli which take a bluish stain are conspicuous and are best seen in lightly stained preparations The cytoplasm is bluish and has a delicate reticular structure. Vacuoles, unevenly distributed fine granules and phagocytized particles may be demonstrable. The cells are often torn in the process of aspiration and smearing (Forteza-Borer: Blood, Special Issue No. 1, July 1947, p. 64; Bayrd, Paulson and Hargraves: Blood *9* 46, 1954)

Reed-Sternberg cells resemble intermediate and mature megakaryocytes in that both cell types are large with voluminous cytoplasm and multilobulated nuclei. The nuclear chromatin of megakaryocytes is pyknotic and nucleoli are not demonstrable. Megakaryocytes have a granular cytoplasm and often have foamy cytoplasmic attachments, marginal vacuoles or platelet formation at the periphery Giant cells of metastatic tumors and the polyploid cells of reticulum sarcoma may have prominent nucleoli and a reticulum chromatin structure, but these malignant cells with abnormally large nuclei usually have a relatively small amount of cytoplasm in contrast to Reed-Sternberg cells which characteristically have an increased amount of cytoplasm

In the majority of patients with Hodgkin's disease it is not possible to find cells of the Reed-Sternberg type, for the granulomatous lesions are not widely distributed in the bone marrow in the early stages of the disease and are unevenly distributed at the time of death. The marrow is usually cellular There is usually a moderate myelocytic and erythrocytic immaturity and an increase in eosinophils, plasmocytes and histiocytes The lymphocytes in the bone marrow and peripheral blood are decreased

Aspiration of an area of granulomatous involvement frequently results in a "dry tap" because of the solid and fixed nature of the tissue Often the examination has to be made on minute portions. In the best smears the Reed-Sternberg cells are few. Type-specific cells are hard to find Varadi (British J. Hematology *1*:184, 1955) recommends that needle puncture of the marrow be performed at a site in which the bone is abnormally tender. The definitive diagnosis of Hodgkin's disease on the basis of morphology of a few cells of the type described above, in the absence of tissue sections and clinical data, is hazardous and is not recommended. The finding of such cells is presumptive evidence and may lead to the consideration of the disease in unsuspected cases

## HISTOPLASMA CAPSULATUM

The diagnosis of histoplasmosis is established by the finding of *Histoplasma capsulatum* within the cytoplasm of phagocytic histiocytes, monocytes and occasionally neutrophils. The organisms appear in the vacuoles of macrophages as round or oval bodies 2 to 5 micra in diameter. In thin smears which are well stained the yeasts have a reddish nuclear chromatin, a juxtanuclear unstained area and a light bluish, finely stippled cytoplasm. These organisms appear within vacuoles and are surrounded by a narrow clear zone or light area, producing a halo effect. In thicker and darker stains the yeasts appear as dark ovoid bodies with no discernible internal structure. The number of organisms found within a given cell or even on a given slide may be quite variable When numerous organisms are encountered, some may be extracellular.

*Histoplasma capsulatum* is to be differentiated from basophilic granules, phagocytized pigment, nuclear fragments and the Leishman-Donovan bodies of kala-azar Phagocytized particles of pigment and cellular debris vary in size and shape and have no differentiation of nucleus and cytoplasm. Many pigment granules have distinctive yellow, brown or black colors. Leishman-Donovan bodies are approximately the same size and shape of the phagocytized yeasts, but have, in addition to the red nucleus and blue cytoplasm, a distinct dark dot or kinetoblast (Plate XXXI).

Macrophages, monocytes or neutrophils containing *Histoplasma capsulatum* may be demonstrable in smears of peripheral blood. Search for them should include the feathered end of the smear and the margins There is greater chance of finding them in smears of aspirated bone marrow or spleen than in peripheral blood smears. Inability to demonstrate the etiologic agent does not exclude histoplasmosis Examination of smears of the buffy coat from peripheral blood or bone marrow may reveal organisms which are not demonstrable by direct examination of the smears Cultures of bone marrow or peripheral blood for fungus are occasionally positive when it is not possible to find the etiologic agent on direct examination.

### LEISHMANIA BODIES

The diagnosis of kala-azar, a disease produced by the protozoal organism, *Leishmania donovani*, may be established by the finding of the organisms with a characteristic morphology in smears of bone marrow and spleen and sometimes in the peripheral blood (Plate XXXI). The organisms are usually contained within the cytoplasm of phagocytic histiocytes, macrophages or monocytes, but may be demonstrable also in the cytoplasm of neutrophils The leishmania bodies are round or ovoid, measuring 2 to 5 micra in diameter. There is a reddish staining nucleus and a blue nongranular cytoplasm. In addition to the nucleus there is a smaller dotlike or rod-shaped body which stains dark blue This structure, known as the kinetoblast, is the anlage for the flagella of the free-swimming flagellate or leptomonas stage The leishmania bodies of kala-azar (*Leishmania donovani*) are morphologically identical to *L. brasiliensis* and *L. tropica* which produce respectively the diseases known as mucocutaneous leishmaniasis (espundia) and dermal leishmaniasis (tropical or oriental sore).

## Metastatic Malignant Cells

Metastatic malignant cells in the smears of bone marrow are best detected by searching under low power illumination, for these fixed tissue cells are usually few and are unevenly distributed They tend to appear in groups which stain more intensely than do the normal marrow cells (Figs 50, 51) Metastatic malignancy should be suspected in any mar-

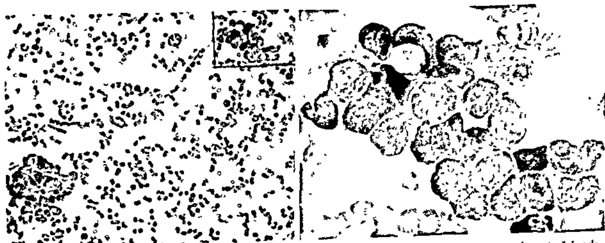


*Figure 50.* Photomicrograph of sternal bone marrow (clinical suspicion of multiple myeloma) Islands of malignant cells Metastatic carcinoma of lung (*Left* Low power, *right* oil immersion )

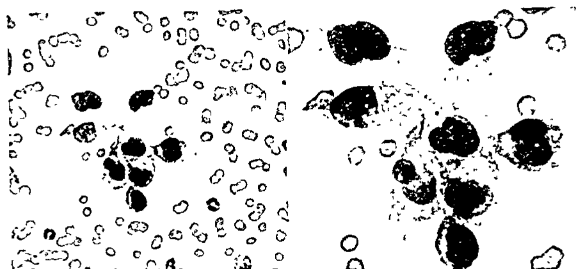


*Figure 51.* Photomicrograph of sternal bone marrow. Amelanotic malignant melanoma. (*Left* × 350. *Right.* × 700.)



*Figure 52.* Metastatic cells in bone marrow smear. Carcinoma of lung (Photograph of painting by M. Guthrie.)

*Figure 53.* Bone marrow Metastatic malignant cells Adenocarcinoma (Photograph of painting by M. Guthrie )

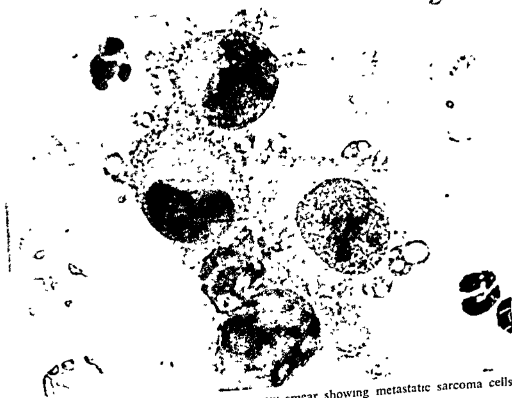


*Figure 54* Photomicrograph of bone marrow smear showing metastatic sarcoma cells

row in which cells are found which are foreign to the marrow and do not fulfill the morphologic criteria of cells usually encountered.

Malignant cells vary considerably in size, nuclear size and size of nucleus in relation to cytoplasm and in the structure and staining characteristics of the nucleus. The nucleus is usually round or oval but may be slightly indented or lobulated. Nucleoli are often demonstrable and may be large in relation to the size of the nucleus. The cytoplasm is usually basophilic and is often unevenly stained. Vacuoles are often present in the cytoplasm (Fig. 52). Granules and phagocytized particles are rarely observed. Variations which favor the diagnosis of malignancy include abnormal mitoses, giant cells, multiple nuclei and particularly nuclei of varying size and structure within the same cell, nuclear clefts and cleavage lines, clumping of nuclear chromatin and uneven staining of the nucleus. Herniation of nuclear material through the nuclear membrane and aberrant nuclear material in the cytoplasm also are presumptive signs of malignancy. Large naked nuclei are often observed in smears of aspirated marrow material in which there are metastatic malignant cells.

In rare instances the cells may be arranged in a glandular pattern, but as a rule they form a syncytial mass or colony of cells without intermingled fat or marrow cells. Mucus-secreting cells may contain globules of lightly stained material which cause the cytoplasm to stain unevenly. Large secretory globules may compress the nucleus and produce a signet-ring effect (Fig. 53). Sarcoma cells often have multiple blunt pseudopods or a cytoplasm which tapers to a point on one or more sides (Fig. 54).

Tumors which most frequently metastasize to the bone marrow are sarcomas and carcinomas of the prostate, breast, thyroid, kidney and bronchus. The morphology of prostatic and breast tumors and reticulum cell sarcoma may closely resemble plasmocytes (see Chap. V).

The incidence of demonstrable metastatic cells in aspirations of the marrow is small, and routine marrow aspirations therefore are not recommended in malignant conditions On the other hand, the marrow may reveal unsuspected malignant cells in conditions in which there is unexplained fever, unexplained cachexia, weight loss and leukemoid peripheral blood pictures In known malignancies, the finding of malignant cells in the bone marrow indicates that the tumor is inoperable. The yield of positive biopsies is increased if the specimen is taken from areas in which there is bone tenderness or pain and in areas in which there is x-ray evidence of bone involvement

*Chapter IX*

# TECHNIQUES AND METHODS

## Blood Smear

### *Cleaning of Slides and Coverslips*

New slides and coverslips which have been specially cleaned are recommended for hematologic study. These slides are washed in warm soapy water, or in a solution of trisodium phosphate or other detergent, thoroughly rinsed with running tap water followed by distilled water, and finally placed in 95 per cent ethyl alcohol. A clean, lint-free cloth should be used for drying slides and coverslips. After drying, the slides may be wrapped in paper packages of four or more and placed in a covered slide box.* Touching the surface of the slide should be avoided. Coverslips should be handled with forceps and after drying placed in a lidded cylindrical box or covered dish.

* Clean, ready for use microscopic slides in coated, lint-free box are now available.

## *Making a Blood Smear*

### SLIDE METHOD

The skin of the fingertip or ear lobe is cleaned with 70 per cent alcohol. The skin is punctured with a sterile needle. A Hemolet (American Hospital Supply Corporation) is recommended. The excess alcohol and the first drop of blood are wiped away with dry sterile absorbent cotton and a second drop is expressed from the wound. The surface of a clean slide is touched to the drop but is not allowed to come in contact with the finger. The drop of blood should be near one end of the slide. Turning the slide over, the end away from the drop is held between the thumb and forefinger of the left hand, with the other fingers serving as a supporting prop. With the right hand another slide is picked up and held loosely between the thumb and forefinger. This spreader slide is placed in front of the drop and brought back until it touches the blood. As soon as the drop spreads almost to each edge, the spreader slide, held at an angle of approximately 30 degrees, is pushed forward. A slow, even motion is employed. Downward pressure is avoided. The thickness of the blood smear is controlled by the size of the drop and the angle of the spreader slide. The greater the angle, the shorter and thicker is the smear. After drying the film in air, the name of the patient may be written in pencil at the thick end.

Oxalated venous blood is used for making blood smears as a screening procedure in many clinics but is not recommended for the differential diagnosis of blood diseases. Oxalated blood is inferior to fresh blood because nuclei are distorted (Fig 36), vacuoles are frequently present and oxalate crystals are present in cytoplasm and nucleus. In order to avoid the use of oxalated blood and at the same time to utilize blood from venous puncture, it is recommended that one small drop of blood from the tip of the needle be placed on several slides and the smears made before the residual blood in the syringe is mixed with oxalate.

A good slide preparation should be margin-free, should occupy one half to two thirds of the slide, and should be thick at one end but thin at the opposite end. "Holes" in the film are indicative of grease on the glass. Long streamers on the ends are caused by chipped or dirty spreaders. The thin area in which the red cells are well separated should be approximately 2 cm. in length and should not be so close to the end as to interfere with the examination when a mechanical stage is used. The principal sources of error are too large a drop of blood, improper angle, or dirty slides. Another source of error is too great a pressure placed upon the spreader slide, causing the larger leukocytes to be pushed to the edges and ends.

After a slide preparation is made and before it is stained, it should not be placed by a radiator, sink or steam bath, for moisture causes the red cells to be hemolyzed. The film should be protected from dust, flies and cockroaches.

### COVERSLIP METHOD

Preliminary to the puncture, coverslips are taken from the container by forceps and placed in a specially prepared rack or in slots cut in a box. New coverslips 22 mm square, No. 1, cleaned in the manner given above for slides, are utilized for making smears. The skin is cleaned with alcohol and punctured with a sterile needle.

The first drop is wiped away with dry, sterile absorbent cotton. Two slips are taken up in the left hand. One slip is held by adjacent corners between the thumb and index finger and the other between the thumb and middle finger With the right hand, express a small drop of blood from the puncture site Grasp the top coverslip by corners with the right hand and touch the drop of blood. Drop this coverslip containing blood diagonally on the other slip. If the coverslips are clean, the blood will spread immediately and without pressure. The drop should be small enough so that the blood will not run to all four edges. Holding the top slip by its projecting

corners between the thumb and forefinger of the right hand, pull the coverslips apart with a smooth motion, using the arm and shoulder muscles and keeping the wrist stiff. Let the slips dry in air. They may be placed on a piece of paper containing the patient's name and the date.

A good coverslip preparation should have at least 8 low power fields in which there are no overlapping of red cells and no artefacts, streaks or holes.

## *Staining a Blood Smear*

Blood films should be stained soon after drying. Dried smears will remain in good condition for several days, but they will gradually undergo degenerative changes.

A suitable rack for staining slides or coverslips may be made by passing straight glass rods through two two-hole rubber stoppers and laying them across a staining pan The top of a cork, which is smaller than the coverslip, may also be used for staining coverslips The staining pan may be placed on the drainboard for convenient emptying and to avoid stain on the table top

Slides are stained with Wright's stain, a modified Romanowsky stain which will bring out the red and blue elements in cells in contrasting colors

### WRIGHT'S STAIN

In order to make up 300 ml of this stain, weigh out 0 5 gm dry powder (certified, National Aniline) and put in a clean dry mortar. Measure 300 ml acetone-free methyl alcohol. Add a small amount of methyl alcohol to the mortar and grind the mixture Pour the liquid portion into a bottle which has a tight stopper or screw-on cap Add a little more methyl alcohol to the undissolved dye, grind, pour off the solution into the bottle, and continue this procedure until 300 ml of methyl alcohol has been added Shake the bottle several minutes each day or two over a period of three weeks Then filter through fine paper into a clean dry bottle. Be sure that all glassware is scrupulously clean and dry

Stock bottles of Wright's stain must be kept well stoppered and away from strong acids and alkalis The stain may be dispensed in small dropping bottles. The dropper in the stain must not be used for water and must never rest on the desk or sink.

Distilled water or buffered distilled water may be employed for diluting the stain and for washing films. It may be placed in large bottles on a shelf above the staining rack with a siphon type of U tube and stopcock.

The *p*H of the diluting water for Wright's stain is 6.6 to 6 8. A crystal of hematoxylin dissolved in a test tube of water serves as a convenient indicator If too acid it will be yellow; if neutral it will be a lavender pink color in two minutes; if too alkaline it will be reddish purple If the diluting water is too basic or too acid, it can be adjusted by adding to the stock distilled water 1 per cent hydrochloric acid or 1 per cent sodium carbonate until the proper staining reaction is obtained.

If a satisfactory stain cannot be obtained with distilled water, it is recommended that phosphate buffered water be substituted. It is prepared as follows· 43 5 ml of M/15 $Na_2HPO_4$ (anhydrous 9 5 gm per 1 ) and 56.5 ml of M/15 $KH_2PO_4$ (9.07 gm per 1.) plus 900 ml of distilled water (*p*H 6 7).

In order to adjust the *p*H of the distilled water, certified buffer tablets in vials of 18 with hermetic wax seals may be purchased (Coleman Instruments, Inc , Maywood, Ill ). One tablet dissolved in 100 ml of water will give a *p*H between 6 5 to 6 8

PROCEDURE Place the slide or coverslip on the staining rack in a horizontal position and completely cover with Wright's stain for two to three minutes Then add distilled water (approximately the same amount as in the first step) until the meniscus extends to the edge of the glass The diluting fluid should be added in equal amounts in different portions to avoid uneven staining intensity.

Leave diluted stain on for eight to ten minutes, the exact time being determined by trial with each new batch of stain. A green metallic sheen indicates proper staining. Bone marrow and peripheral blood smears containing many cells require longer staining time than do thin preparations.

Wash stain off thoroughly with distilled water. Wipe bottom of the slide to remove excess dye. Place slides against a pan or in a slanting position to allow excess fluid to drain off. Dry in air.

Before immersion oil is added, examine under low power of the microscope to note if the stain is satisfactory. If nuclei are not stained, put back on the rack and restain in the same manner as before. If there is excess precipitate, add Wright's stain, and wash immediately with distilled water.

A satisfactory stain is one in which the nuclei of white cells are stained blue with contrasting light cytoplasm, the granules of eosinophils are a brilliant red, and the cytoplasm of red blood cells are a light buff. There should be no precipitate between or on top of the cells.

## *Labeling and Mounting*

All blood films should be labeled by writing directly on the blood film at the time of making the preparation. After staining, a gummed label is placed on one end. The label should give name, date, and type of preparation

Coverslips, after being stained, are mounted by placing a drop of mounting medium (Permount, Fisher Scientific Company) on a clean slide and putting the coverslip, stained surface down, on top of the glass slide The slide then should have a label with appropriate identification

## *Examining the Smear*

For routine examination of blood smears high dry magnification may be used by thoroughly trained workers, but for less experienced examiners and for the study of pathologic blood cells, the oil immersion objective and 10× eyepieces are recommended.

The examination should begin at the thin edge and the slide traversed from side to side. A systematic meander should be followed. A mechanical stage on the microscope greatly aids in performing this count in an orderly fashion. The fields examined should be those in which the red cells are separated and the white cells thinly spread. If the red cells are in rouleaux and the white cells rounded up, this field should be avoided. White cells encountered on the edge of the smear are included in the count.

The examination of the blood smear should not be limited to leukocytes but should include an appraisal of the red cells and an estimation of thrombocytes.

In each microscopic field the size, shape and color content of the red blood cells should be noted Other characteristics, such as diffusely basophilic cells, nucleated red blood cells or parasites should be looked for and, if present, recorded. In peripheral blood the nucleated red cells are usually reported as the number per 100 white blood cells. The stage of development of the nucleated red cells should be classified.

The average number of thrombocytes should be stated. Between 3 and 10 thrombocytes in each oil immersion field are seen on a normal blood smear. In one hundred oil immersion fields 300 to 1000 thrombocytes are usually found and higher counts are not uncommon If platelets appear decreased, then the number per 100 oil immersion fields should be counted.

The different types of white cells seen are recorded as the individual cells are paraded past the field of vision The incidence of each cell is tallied on scratch paper Every white cell encountered should be entered in some column Time may be saved by having a one-unit hand tally counter or a counter with multiple units, such as the 9-unit lab-

oratory tabulator.* When using a single unit counter, record the total number of cells on counter, make tally strokes on paper for all cells except the segmented neutrophils. Arrive at the number of these neutrophils by subtracting the number of all other cells from the total

The identification of some questionable cells by the company they keep is a helpful rule of thumb. If there are quite a few cells which could be monocytes or metamyelocytes, but there is no increase in neutrophilic band cells and there is an increase in monocytes, the questionable cells are monocytes On the other hand, if there is a definite increase in neutrophilic segmented band forms and only a few monocytes, the unknown cells are classified as metamyelocytes or myelocytes. Often the examiner has many cells that he may not be able to classify at first, but after studying several hundred cells and listing the typical cells, he may bridge the gap between the abnormal and the normal cells. The abnormal "unknown" cells are then identified by association with the "known" cells

In many blood smears, there are a few distorted and atypical cells which cannot be classified accurately. If these cells are few in number, it is sufficient to classify them as "atypical" If one suspects that a cell is a lymphocyte, for example, but is not absolutely sure, he may tally this cell with a question mark instead of a straight mark. If the cell is early or abnormal, another symbol might be used to indicate this feature At the end of the differential the examiner should describe the abnormal cells If the morphology of a cell suggests that it lies between two possibilities, make a separate entry, such as "? monocyte—? large lymphocyte" or "? monocyte—? N. metamyelocyte."

One should be hesitant about naming any cell a "blast" unless there are unmistakable morphologic characteristics. If one is not sure, the cell should be reported as "atypical, early."

The minimal number of white cells counted in a differential is 100, but if there are abnormal cells, or a blood dyscrasia is suspected, 200 or more white cells should be studied.

On every blood smear a few smudges are present If there are numerous smudges, these should be tallied while counting the intact cells and reported in terms of number per 100 non-smudged white blood cells.

A suggested form for the reporting of the findings in a blood smear is given in Table 10.

## Bone Marrow Smear

### *Making a Bone Marrow Smear*

Using a syringe as a control and with needle attached, small drops of marrow material are placed on the ends of four to five slides Spreads of these drops are made by a trained technical assistant and preferably by a person skilled in making smears. Then several drops of marrow are placed on a slide, the slide is tilted to allow the more liquid portion to run down, leaving aggregates of marrow or bits of tissue The excess fluid on the slide is reaspirated into the syringe. A "squash preparation" is made by placing another slide on top of the slide containing marrow particles, mashing the slides together and pulling the slides apart in the plane of the surface. These preparations are sometimes useful in detecting malignant cells and megakaryocytes and in revealing the anatomic pattern of the marrow tissue.

Two of the principal errors in marrow procedures are the use of too large a drop of material and improperly spread smears.

* Dr M M Marbel's Blood-Cell Calculator, The Marbel Blood Calculator Company, Chicago, Illinois, Denominator, The Denominator Company, Inc, 261 Broadway, New York 7, N Y, Adams Nine-Unit Laboratory Counter, Clay Adams, N. Y.

**Table 10. Example of Form for Reporting Blood Counts**

UNIVERSITY OF TENNESSEE HEMATOLOGY LABORATORY
MEMPHIS, TENNESSEE
EXAMINATION OF PERIPHERAL BLOOD

Name.................................... ....... ... . . ....... ..........Date.......... ....

......................... ................ ... . .................................. .....

Age. ..M F W C Referred by. . ... ....... . ........

*Red Blood Cells*·

*Thrombocytes:*

*White Blood Cells*· Number counted........

| Normal | | Per Cent |
|---|---|---|
| | Stem Cell | |
| | Myeloblast | |
| | Progranulocyte | |
| | N. Myelocyte | |
| 0–1 | N. Metamyelocyte | |
| 2–10 | N Band | |
| 50–70 | N. Segmented | |
| 1–4 | Eosinophil | |
| 0–1 | Basophil | |
| | Lymphoblast | |
| | Prolymphocyte | |
| 20–40 | Lymphocyte | |
| 1–6 | Monocyte | |
| | Plasmocyte | |
| | Atypical cell | |
| | Disintegrated cells per 100 intact WBC | |

*Summary of Abnormalities*

Interpretation.

Examined by

**Table 11. Example of Form for Reporting Bone Marrow Examination**

UNIVERSITY OF TENNESSEE HEMATOLOGY LABORATORY
MEMPHIS, TENNESSEE
EXAMINATION OF BONE MARROW

Name .... ..... ..... ... ... ... ..... . . . .... ........... ......Date... ..... . ..

Site.. ........ . .. . ....Operator .. ... ... .... ... .......Physician..... ... .. .......

Red Blood Cells

Megakaryocytes·

Thrombocytes:

Nucleated blood cells Number Counted..

| Normal | | Per Cent |
|---|---|---|
| 0–1 | Stem cell | |
| 0–1 | Myeloblast | |
| 1–5 | Progranulocyte | |
| 2–10 | N Myelocyte | |
| 5–15 | N Metamyelocyte | |
| 10–40 | N Band. | |
| 10–30 | N Segmented | |
| 0–3 | Eosinophil | |
| 0–1 | Basophil | |
| 5-15 | Lymphocyte | |
| 0–1 | Plasmocyte | |
| 0–1 | Histiocyte | |
| 0–2 | Monocyte | |
| | Atypical cell | |
| 0–1 | Rubriblast | |
| 1-4 | Prorubricyte | |
| 5-10 | Rubricyte | |
| 10-20 | Metarubricyte | |
| 4 1 | WBC Nucleated RBC ratio | |

Summary of Abnormalities

Interpretation

Examined by

An additional procedure is the sectioning of the remaining clot. The marrow left in the syringe is allowed to coagulate at the top of the partially withdrawn plunger, after which it is removed and put into a bottle containing 10 per cent formalin or Zenker's solution. The marrow clot is then sectioned and stained with hematoxylin and eosin. The combination of a smear study of the aspiration and a fixed tissue preparation of the clot is of much value in the overall evaluation and interpretation of the marrow. If lupus erythematosus is suspected, a portion of the marrow should be heparinized. About thirty minutes after collection it should be placed in a Wintrobe tube and then centrifuged for five minutes. Films of the buffy coat are made.

If surgical biopsy specimens are to be obtained, it is essential to puncture the marrow cavity prior to opening the bony cage and to aspirate marrow for smears in the usual manner. Smears made from the depth of a surgical wound are likely to be grossly contaminated with peripheral blood and to contain few marrow elements.

## *Staining a Bone Marrow Smear*

Bone marrow smears are stained in the manner described for blood smears, allowing a longer staining time. The less perfect slides may be saved for a peroxidase stain, which is helpful in making decisions when the cells are of undifferentiated types

## *Examining a Bone Marrow Smear*

Before placing oil on the smear or mounting under a coverglass, the various slides are examined under low power to select the most suitable slide for counting The distribution of cells, the number of megakaryocytes, any abnormal cell groups, the amount of fat, and other structures which are likely to be few in number are noted

Under oil immersion a differential count of 200 or more nucleated cells is made, and each cell encountered is recorded in its appropriate column. The nucleated red blood cells are included in the percentage of cells. The characteristics of the red cells and thrombocytes are studied. A recommended form with the range of normal values is given in Table 11. Cells such as tissue basophils, mitotic figures, megakaryocytes and macrophages are not included in the myelogram, for these cells occur in such small numbers that numerical evaluation has little significance. However, if these cells are present, a statement should be made. If there is a marked increase in the number of eosinophils or basophils, these cells should be catalogued in the same manner as are the neutrophils

Megakaryocytes are characteristically found at the ends or edges of smears, and therefore all parts of several films should be searched to determine if these cells are present In thrombocytopenic states, a differential megakaryocyte count should be performed.

In reporting results of bone marrow study one should remark whether or not the preparation is satisfactory for appraisal, and give an idea about the degree of cellularity. From a smear alone it is impossible to tell if the marrow is hypocellular or hypercellular. If there are many nucleated cells in every microscopic field and a little fat, it may be stated that the marrow is cellular, but the degree cannot be accurately estimated without a section of marrow tissue. If the finding in marrow and in blood are similar and there are only a few marrow cells, one is unable to tell whether the marrow has been diluted with blood or is hypoplastic. Dilution with blood is an important handicap in interpretation

Mention should be given of any significant abnormalities or unusual features, such as an increased number of mitotic figures, degenerative changes, Auer rods, phagocytized pigment or inclusions. If certain diseases, such as histoplasmosis, malignancy or lupus erythematosus are suspected and the diagnostic cells are not found, then this fact should be mentioned.

A search for islands of malignant cells is a necessary part of a marrow study.

At the time of the marrow aspiration a peripheral blood smear should be made so that the two counts can be correlated

### *Analyzing the Findings*

In the analysis of marrow findings the first step is to calculate the leukocyte to nucleated red cell ratio Normally the ratio is approximately 4 1, with a range of 6·1 to 2:1. A ratio of less than 2:1 indicates an erythrocytic hyperplasia whereas one greater than 6 1 indicates a leukocytic overstimulation or possibly an erythrocytic hypoplasia. A comparison of major cell types, such as neutrophil, eosinophil, lymphocyte, plasmocyte, histiocyte and nucleated red cell, is next made. An eosinophil count above 5 or a lymphocyte count above 20 is probably significant and should be stated as an abnormality. If the marrow is practically replaced by one type of cell, the morphologic features of the cells should be given. This finding is indicative of a leukemic process

After an evaluation of the major cell groups in relation to each other, cells in each family are evaluated for maturity and for signs of degeneration Any other abnormality such as hypersegmentation or asynchronism in maturation of nucleus and cytoplasm should be recorded Analysis of abnormalities should include a terse summary of deviation from the normal pattern

After analyzing the abnormalities an interpretation is given. A bone marrow examination is seldom diagnostic but is often of value in diagnosis

## Reticulocyte Count

The procedure preferred for reticulocyte count is the moist brilliant cresyl blue slide method If material cannot be examined immediately or if permanent preparations are desired, the dry brilliant cresyl blue method is suggested.

### MOIST BRILLIANT CRESYL BLUE SLIDE METHOD

One drop of 1.5 per cent brilliant cresyl blue in 95 per cent alcohol is placed on a clean slide and allowed to dry, leaving a thin deposit of dye. To facilitate even spreading and rapid drying the slide may be previously warmed. A substage lamp will create an adequate amount of heat. The surface is brushed lightly with lens paper to remove excess dry particles. Slides made in this manner may be prepared in quantities and kept in a covered container until needed.

A small drop of blood is taken on a specially cleaned coverslip, and the coverslip with the drop side down is placed on the dried film of dye The drop of blood will spread without pressure if the glassware is clean, but if it does not spread, gentle pressure may be applied. In order to prevent the preparation from drying, the edges of the coverslip may be rimmed with petrolatum (Vaseline). The preparation should stand for approximately ten minutes to allow the dye to penetrate the cells.

In a satisfactory preparation the nuclei and granules of the white cells and the thrombocytes are stained blue and the erythrocytes are yellowish green The reticulated material in the immature red corpuscles (proerythrocytes) will stain deep blue. The distribution, amount and granularity of the granulofilamentous substance varies widely Sometimes only a small number of discrete granules are scattered through the erythrocyte.

An error which is most commonly made in a moist preparation is the use of too large a drop of blood, which causes the red cells to aggregate in such a manner as to make it impossible to count individual cells. If the drop of blood spreads to more than two edges of the coverslip, the preparation is too thick. The red corpuscles should be evenly distributed without overlapping. Other sources of error are an excess amount of dye and precipitated particles adhering to the red corpuscles. An inadequate amount of dye in relation to the number of cells and stainable reticulum is another source of error. Crenated red cells are often mistaken for reticulocytes.

One thousand erythrocytes are counted in successive oil immersion fields, and the number of red cells containing reticulum is recorded. In order to accelerate this procedure a tally counter should be used to record the total number of erythrocytes The reticulocytes may be counted mentally or recorded on paper. The count is reported as the percentage of reticulocytes.

In order to limit the size of the visual field and to increase the accuracy of enumeration, an ocular disk may be placed in the eyepiece. The Miller ocular disk (Bausch and Lomb Optical Company, Rochester, N.Y.) is a suitable one for this purpose. The disk should be placed in a special eyepiece for use with reticulocytes only, so that the ocular will not be unscrewed frequently and dust particles allowed to settle on the lens system

DRY BRILLIANT CRESYL BLUE SLIDE METHOD

A drop of alcoholic brilliant cresyl blue is placed on a slide and spread with another slide The film is allowed to dry in air. A spread of a small drop of blood is made on top of the dried film of stain. In order to retard drying of the slide it is immediately placed in a covered dish or Coplin jar which contains a moistened filter paper This should stand for ten minutes.

After slide has been removed from the moist chamber, the film of blood should be allowed to dry in air and counterstained with Wright's stain After staining, the reticulocytes are counted in the same manner as stated above

The principal advantage of this method is that the count may be performed at a later date, whereas in the wet method the count must be done in a few hours The dry method permits permanent preparations to be made Great care should be used in order to prevent drying of the blood smear before the dye has had sufficient time to penetrate the red cells and stain the granulofilamentous structures.

## Technique for Demonstrating L.E. Cells in Blood (Method of Magath and Winkle*)

Ten milliliters of blood is drawn and placed in a serologic tube. It is allowed to clot for at least two hours at room temperature Rim the clot if necessary and remove the serum. Place wire sieve (American Hospital Supply Corporation, Scientific Products Division) over a Petri dish and turn the clot onto the sieve. Mash the clot through the sieve with a pestle or the bottom of a large test tube. Fill a Wintrobe sedimentation tube with the blood expressed into the Petri dish. Centrifuge at 2000 r.p.m. for five minutes. Withdraw the serum down to the layer of leukocytes. Make smears from the buffy coat of leukocytes. Stain with Wright's stain and examine (see Chap VI).

Immediately after use the sieve should be placed in a pan of soap mixture or detergent for several minutes, washed, rinsed and dried.

## Peroxidase Stain (Method of Sato and Sekiya)

The peroxidase stain depends on the presence of "the peroxidase group to catalyze the transfer of oxygen from hydrogen peroxide and other peroxides to a variety of acceptors" (Gomori *Microscopic Histochemistry*, University of Chicago Press, 1952). Benzidine in the presence of copper sulfate is oxidized to a blue-black compound in the presence of myelocytic granules.

SOLUTIONS

A Aqueous copper sulfate, 0.5 per cent

B. Saturated aqueous solution of benzidine. (Rub in a mortar 0.2 gm benzidine with a few drops of distilled water Add 200

* Am. J Clin Path 22 586 (June) 1952

ml. distilled water and filter. To the filtrate add 4 drops of 3 per cent hydrogen peroxide. Store in a dark place.)

C. Aqueous solution safranin, 1 per cent.

PROCEDURE

Solution A is applied to a dried blood smear for one minute. The copper sulfate solution is poured off. Solution B is added immediately and allowed to remain for two minutes. The film is then washed off with water and counterstained with Solution C for two minutes.

After the smear has dried, it is examined and the staining reaction of 200 nucleated cells is recorded in three categories. (1) heavy granules, (2) few granules, and (3) no granules.

A good stain is characterized by heavy blue-black granules in cells which have segmented nuclei. The erythrocytes are laked and appear as ghosts (Plate XXIV). Neutrophilic and eosinophilic granules give a strongly positive reaction in their more mature stages and a less strong reaction in the immature forms There is a difference of opinion about the reaction of basophilic granules. Monocytes generally have a few blue-black granules but often give a variable reaction.. Lymphocytes, plasmocytes, nucleated red cells and undifferentiated (blast) cells react negatively and are stained red. Immature myelocytic cells without granules or with nonspecific granules in the cytoplasm as revealed by the Wright's stain may give a positive peroxidase stain Herein lies the main use of the peroxidase stain—that of differentiating lymphocytic leukemia from myelocytic leukemia of immature type. The stain is of little value in the diagnosis of stem cell leukemia or in telling the difference between early monocytic and early myelocytic cells.

## The Detection of Sickled Erythrocytes

SEALED MOIST PREPARATION

One small drop of blood is placed on a coverslip. The coverslip is put drop side down on a slide. The drop is allowed to spread without pressure The edges of the coverslip are then sealed with petrolatum (Vaseline) or other semisolid oil to exclude air. The sealing material should not be placed under the coverslip for this makes the preparation too thick. Blood from a skin puncture wound or fresh venous or oxalated blood may be used. It is preferable to make at least two preparations. Care must be taken to avoid mixing the blood with alcohol or disinfectant used in cleaning the finger. Scrupulously clean slides and coverslips are necessary, for residual films of soap or detergent, acid or other substances may interfere with metabolism of the living cells and bacteria and prevent the uptake of oxygen which is essential to create the conditions of hypoxia upon which the test is dependent. Examination for sickling of the erythrocytes under high dry magnification is made If negative, the smears are left at room or incubator temperature for twenty-four hours before making the final reading Overheating or overcooling are to be avoided. The morphology of the multipointed cells, called "sickled cells," is discussed in Chapter IV

A refinement of the moist preparation is the collection of capillary blood during venous stasis In this procedure a rubber band is placed around the proximal phalanx of a finger for three to five minutes. The concentration of oxygen is reduced when the venous circulation is obstructed by the rubber band, thus favoring the crystallization of hemoglobin. The skin is punctured and a drop of the hypoxic blood is taken up on a coverslip and

immediately placed on a slide. The edges of the coverslip are rimmed with Vaseline. These preparations, like those made of aerated blood, should be kept for twenty-four hours before reporting the blood as negative for sickled erythrocytes. The presence or absence of hemoglobin S can be established by electrophoretic methods.

### SODIUM METABISULFITE METHOD

A small drop of fresh capillary or venous blood is mixed with a relatively large drop of freshly prepared 2 per cent sodium metabisulfite. The sodium metabisulfite is a reducing agent which causes the abnormal hemoglobin S to become insoluble and to form crystals within the erythrocyte, resulting in the multipointed and fish-fin forms known as sickled cells (Chap. IV). Tablets of sodium metabisulfite specially prepared for sickle cell testing are now commercially available (A. S. Aloe and Co., St. Louis, Mo.). One tablet dissolved in 10 ml. of distilled water gives the proper concentration. It is necessary to make up the test solution fresh each day because it is unstable and deteriorates rapidly on standing. Erythrocytes containing hemoglobin S are usually demonstrable in appreciable numbers within a few minutes after mixing the blood with the reducing agent. It is best to reexamine again at the end of thirty to sixty minutes if the preparation is negative immediately or if there is doubt about the specificity of the pointed forms. If possible, control tests should be performed. The errors of this and other sickle cell methods are mostly due to the failure to detect the presence of sickle cells when hemoglobin S is present, but inexperienced workers may mistake crenation or poikilocytosis of a nonspecific type for sickled cells.

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